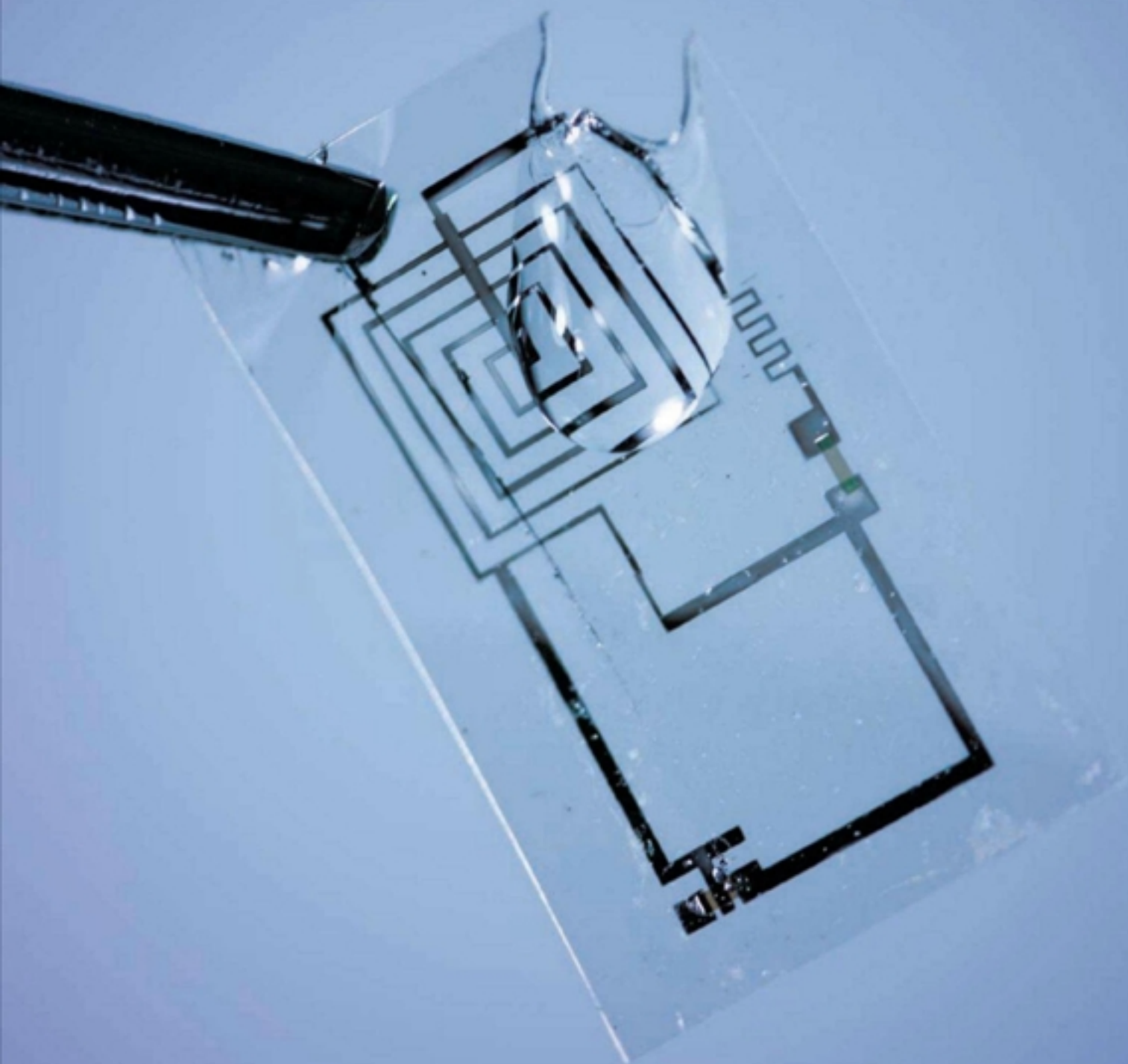


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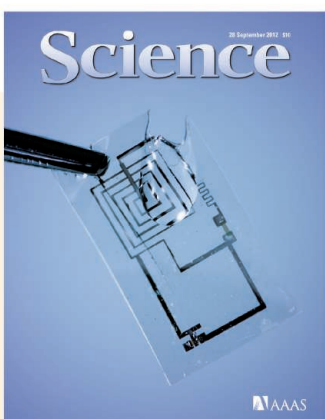
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COVER

A biodegradable integrated circuit (length: ~2.54 centimeters) shown partially dissolved by a droplet of water. This demonstration system includes transistors; diodes; inductors and capacitors, with magnesium for the electrodes/interconnects; magnesium oxide for the gate/interlayer dielectrics; silicon nanomembranes for the semiconductor; and silk as a thin, flexible substrate. The dissolution characteristics can be tuned to address applications such as bioresorbable implants, biodegradable environmental monitors, and compostable consumer electronics. See page 1640.

Image: Beckman Institute, University of Illinois, and Tufts University

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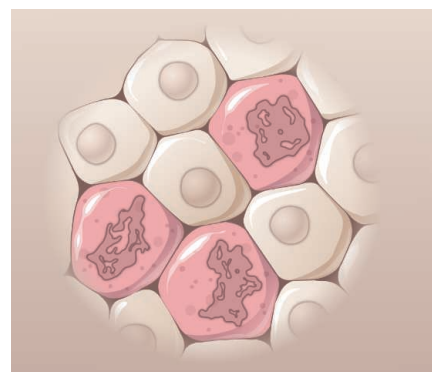
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10.1126/science.1223389

Coagulation Factor X Activates Innate Immunity to Human Species C Adenovirus

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A study of 16th to 19th century eunuchs suggests that testosterone may shorten men's lives.

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Form Biofilm Folds

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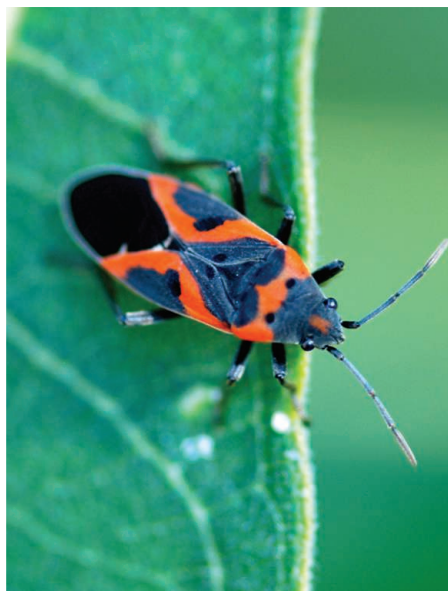
Melt-away electronics, scientific thinking in kids, climate change in Mongolia, and more.

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ADVANCING SCIENCE, SERVING SOCIETY



<< Making the Change

The Apocynaceae plant family produces toxic cardenolids. However, many insects have managed to escape the deleterious effects of these chemicals and even, in some cases, use them in their own defense. **Zhen *et al.*** (p. 1634) investigated a broad range of taxa and found several examples of parallel changes, as well as duplications, in members of the ATP α family that likely explain the shift to allow insects to avoid the toxic effects of these plants. Thus, natural selection can harness a combination of gene duplication, protein evolution, and regulatory evolution to allow distantly related species to adapt to specific niches.

Kindergarten Scientists

Becoming a scientist takes many years of training . . . or so it seems. It requires learning how to acquire data, to incorporate it into hypotheses, and then to test those hypotheses against newly acquired data; it requires learning how to think critically and to apply statistical analysis. **Gopnik** (p. 1623) reviews recent cognitive development findings that demonstrate how preschoolers act as young scientists in refining their intuitive representations of the world as they explore reality.

Changing Rains

The water cycle of the western United States has varied dramatically across the glacial cycles of the Pleistocene, possibly because of changes in the tracks of the storms that deliver moisture to the region. **Lyle *et al.*** (p. 1629) present evidence from a collection of Great Basin lakes which show that water levels rose over the last 20,000 years because of moisture transported from the tropical Pacific, not from a southward diversion of the westerly storm track. Furthermore, the timing of the lake level highs in the Great Basin shows a progression from south to north that does not coincide with the northward progression of wet intervals.

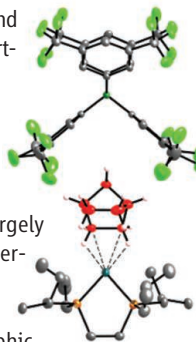
Our Constant Sun

The exact shape of the Sun provides information on its internal structure. Based on data obtained by the Helioseismic and Magnetic Imager aboard NASA's Solar Dynamics Observatory, **Kuhn *et al.*** (p. 1638, published online 16 August; see the Perspective by **Gough**) measured the solar shape

during a 2-year period in which the Sun evolved from a minimum to a maximum of sunspot activity. Against expectations, the Sun's oblate shape was found to be constant and not to vary with the 11-year solar cycle.

Solid View of a Sigma Complex

For decades, it has been clear from kinetic studies that saturated hydrocarbons can also act as weak ligands, often just prior to bond cleavage reactions. These short-lived intermediates—termed " σ complexes" because the donated electrons reside in single (sigma symmetry) C-H bonds—have been glimpsed spectroscopically but have largely eluded full structural characterization. **Pike *et al.*** (p. 1648, published online 23 August) now present the crystallographic characterization of an alkane bound to rhodium, which they captured by direct hydrogenation of a more stable crystalline precursor incorporating an alkene.



Reversible Implants

Silicon electronics are generally designed to be stable and robust—it would be counterproductive if the key parts of your computer or cell phone slowly dissolved away while you were using it. In order to develop transient electronics for use as medical implants, **Hwang *et al.*** (p. 1640, see the cover) produced a complete set

of tools and materials that would be needed to make standard devices. Devices were designed to have a specific lifetime, after which the component materials, such as porous silicon and silk, would be resorbed by the body.

Gently Coupled

Linked aryl rings are found in a broad range of commercial chemical products. Currently, the most versatile synthetic route to this motif involves cross-coupling of one ring with a halide substituent to another ring with a boron or metal-based substituent. Recent research has focused on eliminating the need for one or both of these activating substituents, but for the most part, the emerging methods have required high temperatures and high concentrations of one coupling partner. **Ball *et al.*** (p. 1644) now present a gold catalyst that can couple silyl-activated arenes to unactivated arenes in comparable concentrations at room temperature.

Beyond Youthful Aggression

Two decades ago, the combination of a demographic bulge of young males in Enga Province of Papua New Guinea and more deadly armaments triggered a series of intertribal wars and a surge in war-related fatalities; more recently, both wars and deaths have dropped. Drawing upon 20 years of conflict data, as well as historical records of the local social institutions, **Wiessner and Pupu** (p. 1651) analyzed the impact of youth-driven violence upon the traditional hierarchies and the subsequent evolution of the indigenous institutions into political structures capable of controlling conflict and reducing bloodshed.

You Snooze, You Lose

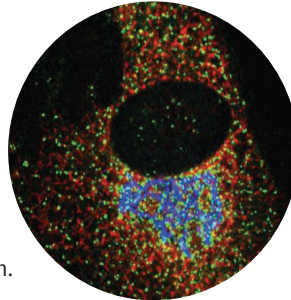
Sleep serves restorative and memory functions, but it does not always operate analogously across species. Deferral of sleep may be possible when selection strongly favors the awake. **Lesku *et al.*** (p. 1654; see the Perspective by **Siegel**) show that sleep may be deferred without cost or impairment in pectoral sandpipers. These birds breed collectively in the high Arctic, and male competition is intense. Competing for, and displaying to, females are both physically and cognitively demanding, yet birds who slept the least showed no decrease in their ability to perform these activities. Indeed, those males who slept the least obtained the most matings and sired the most offspring.

Limiting Your Options

Many species are dependent on specific resources that may limit their range of hosts to that of a few or even a single species. The restriction of the fly *Drosophila pachea* to senita cactus is a classic case of an ecological specialization. **Lang *et al.*** (p. 1658) reveal how multiple amino acid substitutions in the *neverland* gene have rendered *D. pachea* dependent upon the lathosterol of the senita cactus. Thus, relatively few genetic changes can play a large role in determining a species' ecological niche.

A Tight Squeeze

During intracellular transport, the export of procollagen from the endoplasmic reticulum is intriguing because procollagen is too large to fit into conventional coat protein complex II (COPII)-coated transport vesicles. Recent work has implicated the receptor TANGO1 in procollagen export. Now, **Venditti *et al.*** (p. 1668) report that TANGO1 recruits Sedlin—also known as TRAPPC2, a homolog of the yeast TRAPP subunit Trs20—and helps to allow COPII-coated carriers to grow large enough to incorporate procollagen.



A Radical Mechanism

The bacterial enzyme nitrogenase plays a key role in the global nitrogen cycle by catalyzing the reduction of N_2 to ammonia. At the heart of the enzyme is a metal-sulfur cluster that contains an interstitial light atom recently identified as a carbide. The identification raised questions concerning the role of the carbide in the enzyme mechanism and how it is inserted into the metal cluster. **Wiig *et al.*** (p. 1672; see the Perspective by **Boal and Rosenzweig**) now show that the carbide derives from *S*-adenosylmethionine (SAM) and is inserted into the core by the radical SAM enzyme NifB.

No More Junk DNA

The majority of the human genome is transcribed at some level, but the function and evolutionary constraint among regions that do not regulate gene expression or code for genes are generally unknown. Examining data from the 1000 Genomes Project and ENCODE, **Ward and Kellis** (p. 1675, published online 5 September) examined regions of the human genome that did not show conservation among mammals and found evidence for a human lineage-specific constraint spanning approximately 4% of the genome that is biochemically active but not directly associated with genes. Interestingly, these regions showed lower numbers of single-nucleotide polymorphisms, heterozygosity, and derived allele frequencies, suggesting that they are under constraint and may be functional.

Keeping Cancer Cells At Bay

Cancer cells are often aneuploid; that is, they have an abnormal number of chromosomes. But to what extent this contributes to the tumorigenic phenotype is not clear. **Senovilla *et al.*** (p. 1678; see the Perspective by **Zanetti and Mahadevan**) found that tetraploidization of cancer cells can cause them to become immunogenic and thus aid in their clearance from the body by the immune system. Cells with excess chromosomes put stress on the endoplasmic reticulum, which leads to movement of the protein calreticulin to the cell surface. Calreticulin exposure in turn caused recognition of cancer cells in mice by the host immune system. Thus, the immune system appears to serve a protective role in eliminating hyperploid cells that must be overcome to allow unrestricted growth of cancer cells.

Tuberculosis Vaccine Conundrum

Some children experience severe clinical disease when they are vaccinated against tuberculosis, an attenuated live vaccine that is normally innocuous in humans. Several germline mutations have been identified that account for this susceptibility, and now **Bogunovic *et al.*** (p. 1684, published online 2 August) add another to the list—*ISG15*. Uncovering this mutation, which is inherited in an autosomal recessive manner, was a surprise because studies with mice deficient in *ISG15* showed enhanced susceptibility to some viral, but not bacterial, infections. Nevertheless, patients lacking *ISG15* were not able to produce adequate amounts of interferon- γ , a cytokine critical for clearance of the bacteria.



Bruce Alberts is Editor-in-Chief of *Science*.

The End of “Small Science”?

I AM PROMPTED TO WRITE THIS EDITORIAL BY THE RELEASE OF 30 PAPERS THIS MONTH FROM THE ENCODE Project Consortium. This decade-long project involved an international team of 442 scientists who have compiled what is being called an “encyclopedia of DNA elements,” a comprehensive list of functional elements in the human genome. The detailed overview is expected to spur further research on the fundamentals of life, health, and disease. ENCODE exemplifies a “big-science” style of research that continues to sweep the headlines, and the increased efficiency of data production by such projects is impressive. Does this mean that the highly successful “small-science” era of biological research will soon be over? Will government funding increasingly favor big-science projects? I certainly hope that the answer is no.

The striking success of the Human Genome Project, which culminated in the 2004 publication of a near-complete sequence of the more than 3 billion nucleotides of DNA in human chromosomes, has stimulated the proliferation of other large “-omics” projects in biology, including proteomics, transcriptomics, epigenomics, and metabolomics. Each of these big-science efforts drives the development of valuable new methodologies, as required to bring each type of investigation to scale. But the scale also creates a constituency that makes these projects difficult to stop, even when there are clear signs of diminishing returns. In this time of very tight resources, it becomes increasingly critical to make objective, tough decisions about what kinds of projects stand the best chance of producing the results needed for deeply understanding, rather than merely describing, biological systems.

What remains to be understood? As a coauthor of a textbook in cell biology that is updated at 5-year intervals, I am painfully aware of the huge gap that remains in our understanding of even the simplest cells. Consider, for example, the common bacterium *E. coli*, which served as a predominant model organism in the early years of molecular biology. It is very sobering to report that more than 50 years later, nearly a quarter of the more than 4000 proteins encoded by its genome have functions that remain unknown. Might some new functional classes of biological molecules, common to all cells, be discovered by a focus on such proteins?

As a second example, a typical human cell contains approximately 10,000 different proteins, organized into hundreds of different complexes that function as “protein machines,” most being activated where and when needed to perform a specific function such as DNA repair or signal integration. To make sense of the biology and gain the likely health benefits from such an understanding, each of these protein complexes will need to be studied in detail by biochemists—work that typically takes place in small laboratories.

As a final point, we now know that the most interesting properties of cells are “emergent” properties, resulting from elaborate networks of interactions between many different molecules that include the protein machines. Scientists currently lack the ability to decipher this complexity, and gaining this ability will require great ingenuity and many new developments whose exact nature is unpredictable. Much of the work will need to be done through small-science research in relatively simple systems, such as the *E. coli* bacterium, with the hope that what is learned will lead to new approaches and principles that can be transferred to the more complicated cells of mammals.

Each year, the amount of factual information that scientists acquire about cells increases and, stimulated by -omics projects, the compilations of data expand at a tremendous rate. But the grand challenges that remain in attaining a deep understanding of the chemistry of life will require going beyond detailed catalogs. Ensuring a successful future for the biological sciences will require restraint in the growth of large centers and -omics-like projects, so as to provide more financial support for the critical work of innovative small laboratories striving to understand the wonderful complexity of living systems.

— Bruce Alberts



ECOLOGY

Conservation Calculation

Conservation of biodiversity costs money. Not only do the long-term preservation and/or restoration of habitats require investment, the acquisition of lands may be required too. Priority areas for conservation tend to be designated on the basis of their biodiversity value (e.g., the range of species present or the degree to which species and habitats are threatened) or their ecosystem services value (e.g., forest cover or water quality), but the economic costs and the return on investment (ROI) in terms of wider conservation goals are often given less attention. To address this gap, Withey *et al.* developed an economic model aimed at maximizing the ROI on the cost of the acquisition of lands for conservation, using vertebrates in the United States as the target of their analysis (though it could be applied to other organisms and regions too). Their model, which addresses the contribution that local land acquisition can make to vertebrate conservation at the wider regional scale, takes into account factors such as land costs, threats of land conversion for development, species richness, and the proportion of species ranges that are already protected elsewhere. Although local initiatives remain fundamentally important to conservation efforts, this approach can improve on the effectiveness of conservation that is based solely on local species richness or cost. — AMS

Ecol. Lett. **15**, 10.1111/j.1461-0248.2012.01847.x (2012).

CELL BIOLOGY

Recycle to Survive

The proteasome is essential for the controlled degradation of proteins in all cells. Although it is well established that proteasome inhibition is lethal, the underlying mechanism is less clear. Working in yeast, mammalian cells, and flies, Suraweera *et al.* found that proteasome inhibition caused a critical shortage of amino acids, leading to cell death. Cells could cope with high levels of undegraded proteasome substrates, but not with the resulting depletion of key amino acids. The lethality of proteasome inhibition was rescued upon amino acid supplementation. Furthermore, the shortage of select amino acids induced autophagy when the proteasome was inhibited, linking proteasome impairment and autophagy induction. Thus, the proteasome serves a basic housekeeping function as a recycling machine, beyond a simple waste disposal system, and it is the lack of critical amino acids that kills cells when the proteasome is compromised, not the accumulation of protein waste. — SMH

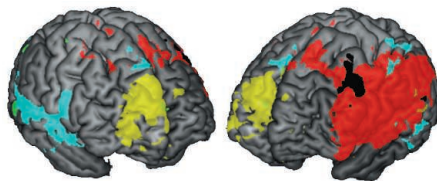
Mol. Cell **47**, 10.1016/j.molcel.2012.08.003 (2012).

NEUROSCIENCE

Mapping the Prefrontal Cortex

Although the human brain's prefrontal cortex (PFC) has been studied for decades, theories about a valuation network and a cognitive

control network—both hypothesized to reside in the PFC—have only recently emerged, and their precise distinction is still unclear. Furthermore, cognitive control, once considered a unitary construct, is now thought to fractionate into distinct executive functions whose neural correlates remain elusive. It is thus still an unanswered question how these processes map onto distinct or possibly overlapping sectors of the PFC. Glaescher *et al.* applied several new statistical mapping approaches to a sample of 344 lesion patients that had received an array of neuropsychological tests of executive functions and value-based decision-making. Background data regarding IQ, memory, and other cogni-



tive functions within individual subjects were also analyzed. The authors described detailed maps of PFC regions that are essential for different executive functions. One set involving the dorsolateral PFC and the anterior cingulate cortex is associated with a common performance factor related to flexibly switching between task and response sets, a hallmark of cognitive control. Another set involving the orbitofrontal

cortex, ventromedial PFC, and frontopolar cortex is involved in value-based decision-making. This study details the essential neuroanatomical substrates of some of the highest brain functions and provides insights about the extent to which they are distinct or overlap. — PRS

Proc. Natl. Acad. Sci. U.S.A. **109**, 14681 (2012).

VIROLOGY

An Atlas of Epstein-Barr Virus

Epstein-Barr virus (EBV), which has been associated with B cell lymphomas, gastric carcinomas, and nasopharyngeal carcinoma, may be responsible for ~1% of all human cancers. Arvey *et al.* have pooled data from nucleosome positioning maps and viral protein-binding analyses with more than 700 publicly available high-throughput sequencing data sets from human lymphoblastoid cell lines to generate a large-scale functional genomics atlas of the virus. Although much of the data was already publicly available, it was scattered, and has now been integrated in a highly usable form. Their analysis revealed possible regulatory domains within the viral genome and combinatorial control of viral gene expression by human transcription factors. There were also indications of three-dimensional organization, including loop formation between the viral origin of latent replication and latent membrane proteins 1 and 2, linked by human transcriptional repressor CTCF and cohesin. B cell specificity factor

Pax5 was shown to bind to EBV terminal repeats, and depletion experiments showed that Pax5 is involved in the regulation of EBV transcription during latent infection. — BJ

Cell Host Microbe **12**, 233 (2012).

OCEAN SCIENCE

A Drop in the Ocean

Observations from over the past 130 years show that global mean sea level has been rising by an average of about 1.7 mm/year and by about 3 mm/year over the past 20 years. Projections of future global mean sea level essentially all agree that this rise will continue as climate warms, mostly because of increasing ocean volume caused by the melting of glaciers and ice sheets, as well as the volume increase due to the thermal expansion of the global ocean. Superimposed on these trends are other variations, both positive and negative, caused by large-scale ocean-atmosphere processes such as the El Niño–Southern Oscillation. Boening *et al.* report that global mean sea level dropped by 5 mm during 2010 and 2011 because of an increase in terrestrial water storage caused by changes in global precipitation patterns accompanying the transition from El Niño conditions in 2009–2010 to a strong La Niña in 2010–2011. The increase in terrestrial water storage occurred mostly in Australia, northern South America, and Southeast Asia. This drop will probably be temporary, though, as runoff to the ocean moves that water back from where it came. — HJS

Geophys. Res. Lett. **10.1029/2012GL053055** (2012).

ASTRONOMY

A Positive Influence

It is thought that most, if not all, large galaxies host massive black holes at their centers. These black holes become luminous when they accrete matter from their immediate surroundings, and during these phases of active accretion, they are thought to influence the evolution of their galaxies' stellar populations (see Volonteri, Special Issue Review, 3 Aug. 2012, p. 544). Whether this influence is positive or negative (i.e., whether black hole accretion suppresses or induces star formation) is a matter of current debate. By combining observations obtained with the European Space Agency's Herschel Space Observatory with data from NASA's Spitzer Space Telescope, Barthel *et al.* inferred the star formation rates for three extremely massive galaxies, whose central

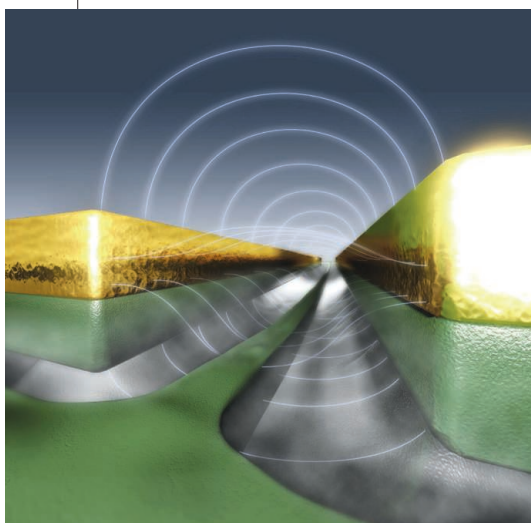
massive black holes are being fed at a high rate. Analysis of these galaxies' spectral energy distributions implies that they are all forming stars at exceptionally high rates, showing that powerful black hole accretion and vigorous star formation can occur simultaneously, at least in the most massive galaxies. — MJC

Astrophys. J. **757**, L26 (2012).

APPLIED PHYSICS

Getting Resonators on Q

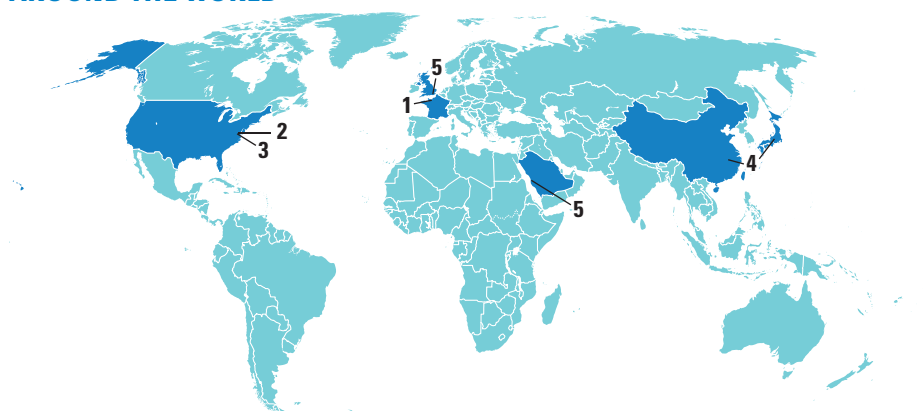
Micro- or nanomechanical resonators find application in a broad range of devices, from mass and chemical sensors to mirror arrays that can dissect and direct multiple light beams to specific targets for adaptive optics and optical communication. Their mechanical response,



however, tends to be fixed by their geometry. Often, the application is to a spectrum of chemical or biological species that may be sampled, requiring a range of frequencies to be measured. That then requires an array of resonators, structured rather like the strings on a harp of different geometry with varied length and thickness, and tuned to a specific frequency. Although some tunability of a single resonator has been demonstrated via electrical actuation, the frequency range of tunability is made at the expense of sensitivity of the resonator (as measured by the Q factor). Rieger *et al.* present a technique based on dielectric actuation and microwave readout for a single silicon-nitride string and show that the resonant frequency can be tuned over a relatively large frequency range and yet still maintain high Q factors. A single-stringed (micromechanical) instrument with the function of a multistringed harp would offer simplicity in device design. — ISO

Appl. Phys. Lett. **101**, 103110 (2012).

AROUND THE WORLD



Caen, France 1

France, E.U. to Review Controversial GM Food Study

A study purporting to show that genetically modified (GM) corn can cause tumors and death in rats was panned by many scientists, but it is nevertheless having a political impact.



A team headed by Gilles-Eric Séralini of the University of Caen reported in *Food and Chemical Toxicology* that rats fed Monsanto's herbicide-resistant maize variety NK603 for 2 years died earlier than rats on a non-GM maize diet, developed more tumors, and

suffered from hormone imbalances.

Critics have said that the study has serious statistical and other problems. But those criticisms received relatively little attention in France, in part because the team provided some French journalists with advance access to the paper but barred them from showing it to other scientists. As a result, the publication was widely reported but few critical notes were sounded until the next day.

Two French agencies and the European Food Safety Authority in Parma, Italy, have been asked to look into the study, and in California, supporters of a proposed law to make labeling of GM food mandatory have seized on it to bolster their case.

<http://scim.ag/GMOstudy>

Washington, D.C. 2

STEM Visa Bill Defeated

The U.S. House of Representatives last week defeated a Republican plan to make it easier for foreign-born students earning advanced science and engineering degrees from U.S.

universities to remain after graduation.

The proposal, by Representative Lamar Smith (R-TX), would have created a new employment-based visa category for up to 55,000 such graduates each year. It would have held overall immigration flat by eliminating a lottery-based program that awards a similar number of visas. Supporters say that kicking out graduates in science, technology, engineering, and mathematics (STEM) fields encourages them to go home and compete against U.S. companies.

Democrats agree that foreign-born STEM graduates have valuable skills, but they also want to preserve the lottery program. They object to including for-profit institutions in the pool of eligible degree-granting institutions and discarding unused slots rather than adding them to another visa category.

The vote (257 to 158) fell short of the two-thirds majority needed for passage under House rules that allowed the bill to be fast-tracked. But the tally, which included 30 Democrats voting yes, allows each side to claim victory on an issue likely to come up again in the next Congress.

http://scim.ag/STEM_visa

Richmond 3

Mann Wins Latest Climate Court Battle

Climate scientist Michael Mann of Pennsylvania State University, University Park, won an initial victory in his legal battle against the American Tradition Institute, a group trying to force the release of his e-mail correspondence while a professor at the University of Virginia in Charlottesville.

On 17 September, a judge in Prince William County in Virginia ruled that the group did not have a right to see Mann's e-mail under the state's freedom of information



laws. Although Mann's correspondence was considered public record, the judge ruled that an exemption protecting data, records, or information produced by faculty or staff members in the course of conducting research applied to the climate scientist's e-mail. The ruling could still be appealed to a higher court.

This latest victory comes on the heels of another battle in March, when Virginia's Supreme Court tossed out an effort by the state's conservative attorney general, Kenneth Cuccinelli II, to compel the University of Virginia to turn over detailed records related to Mann's work. http://scim.ag/Mann_court

East China Sea 4

Dispute Over Islets Threatens Scientific Exchanges—Again

A long-simmering spat between China and Japan for sovereignty over a group of uninhabited islets in the East China Sea is once again denting scientific cooperation between the two nations. The Japanese government's 11 September decision to buy three of the islets from their Japanese owners sparked anti-Japanese demonstrations in China and saber rattling on both sides. In a 15 September online statement, the China Association for Science and Technol-



ogy, an umbrella organization representing more than 160 professional societies, called the purchase "a gross violation of China's territorial sovereignty" that "hurt the feelings of the Chinese people and undermined bilateral relations," China's Xinhua news agency reported. The Chinese asked

to postpone a China-Japan University Fair and Forum scheduled for the end of September. They also suspended a plan to donate two rare crested ibises to a Japanese captive-breeding program. http://scim.ag/China_Japan

Jeddah, Saudi Arabia, and London 5

Health Officials on Alert Over New Coronavirus

Public health experts are on edge about a new virus discovered in two patients, one of whom has died. The new infectious agent is a coronavirus, a group whose members include four viruses that cause the common cold as well as the agent that causes SARS—a severe respiratory syndrome that killed more than 700 people and sickened more than 8000 in a global outbreak in 2002 and 2003.

The first known patient was a 60-year-old man from Jeddah, Saudi Arabia, who died of pneumonia in July; his case was reported by Ali Mohamed Zaki of the Soliman Fakeeh Hospital in Jeddah on ProMED, an e-mail list about emerging infections, on 15 September. On 23 September, the United Kingdom's Health Protection Agency reported that a 49-year-old Qatari man treated for a severe respiratory syndrome in a London hospital was infected with the same virus. Several other cases are still under investigation this week. It's unclear whether the virus can be transmitted between humans or how big a threat it may pose to public health. <http://scim.ag/coronavirus>

NEWSMAKERS

Three Q's

On 13 August, cardiologist **Gary Gibbons**, 55, became director of the \$3 billion National Heart, Lung, and Blood Institute (NHLBI). Gibbons comes to NHLBI from Morehouse School of Medicine in Atlanta, where he directed a cardiovascular research center.



Gibbons

Q: Why were you interested in this job?

I've always been motivated to address scientific questions that have implications for patients and patient care and public health. And I have a particular passionate commitment to expanding the diversity of the biomedical workforce. This position gives me an opportunity to really pursue that goal.

Whirlpool Galaxy Takes Center Stage

M51—known as the Whirlpool galaxy—is a classic spiral galaxy that astronomers have studied for centuries. But this mesmerizing new image has nabbed Australian photographer Martin Pugh the top prize in the fourth annual Astronomy Photographer of the Year awards for the second time. He won Astronomy Photographer of the Year in 2009. The galaxy portrait—noted for its sharp detail of the spiral's arms, defined by dark, dusty areas and bright, pink clouds of hydrogen—is on display at the Royal Observatory, Greenwich, in the United Kingdom from 20 September until 17 February 2013. Visitors can also view winners in categories such as "Young Astronomy Photographer of the Year," "Earth and Space," and "Our Solar System," alongside Pugh's winning shot. Pugh's photograph, chosen from more than 800 entries, also earned him £1500. http://scim.ag/astro_photo



Q: What motivates you to pursue public service?

One of my heroes growing up was my mother, who exemplified this notion of community service, giving back. That's derived from her own personal experience as an orphan growing up in the Great Depression. I'm carrying on in that sort of family tradition.

Q: What are the big challenges ahead?

We tend to think of [heart, lung, and blood diseases] as chronic diseases. And yet what we're learning in areas of reparative biology, in terms of epigenomics, these advances suggest ways of intervening that can kind of erase or reboot the memory of the body and change the natural history [of these conditions].

We often get jealous of our oncology colleagues [who] talk about the remission of the disease. We're not quite there, but those would be frontiers that we'd be excited to pursue. http://scim.ag/Gary_Gibbons

Princeton President Shirley Tilghman Steps Down

Princeton University president and molecular biologist **Shirley Tilghman** will resign in June after leading the university for 12 years.

"There is a natural rhythm to university presidencies," Tilghman wrote in a

resignation letter on 22 September to the Princeton community. With a \$1.9 billion fundraising campaign completed and her



Tilghman

priorities accomplished or on their way, she wrote, "it is time for Princeton to turn to its 20th president to chart the path for the next decade and beyond."

Tilghman gave up her research on mammalian genetics to become Princeton's first woman president. She worked to increase the number of students receiving financial aid, created a neuroscience institute, recruited new chemistry faculty members, and launched energy and environmental programs. >>

NOTED

>The **first-ever paper about XMRV**, linking the controversial virus to prostate cancer, **has been retracted**. As *Science* reported last week (21 September, p. 1441), authors of the 2006 study in *PLoS Pathogens* had shown it contained errors but said they did not want to retract it. By the time *Science's* story appeared, the editors of *PLoS Pathogens* had already retracted the paper without the authors' consent. http://scim.ag/XMRV_retract

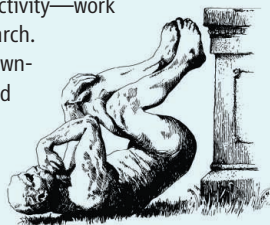
Random Sample

Ig Nobels to Studies of Swaying Ponytails, Dead Salmon

"I used to jog around the Stanford campus and saw many young ladies running," Joseph Keller told a packed theater on 20 September in Cambridge, Massachusetts. "Their ponytails swayed side to side like this"—the Stanford University mathematician tossed the dangling tassels of a fez hat—"even though the head was only going up and down. Why did the ponytail go side to side?" For finding a mathematical explanation, Keller and a team of physicists scooped one of the coveted 2012 Ig Nobel prizes.

Awarded each year in a lighthearted ceremony a few weeks before the actual Nobel prizes are announced, the Ig Nobels celebrate "research that makes people laugh, and then think." This year, for example, a team led by Craig Bennett of the University of California, Santa Barbara, grabbed the neuroscience prize for "proving" that a dead salmon has brain activity—work that highlighted a common statistical pitfall in brain imaging research.

This year's recipients had to contend with a giant inflatable clownfish drone that hovered over the stage, colliding with walls and people alike, and with the SpeechJammer, a device that thwarts people from speaking by projecting their voice back at them with a tiny delay. The invention won two Japanese researchers this year's Ig Nobel prize in acoustics. http://scim.ag/_lg2012



BY THE NUMBERS

5 years and 3 years Amount by which life expectancy of white U.S. women and men (respectively) without a high school diploma decreased between 1990 and 2008, according to research published in the August issue of *Health Affairs*.

110 Number of chimpanzees that the U.S. National Institutes of Health will retire from its Louisiana research center by August 2013.

179 Number of new Thailand wasp species identified by an international team of researchers. Scientists named 34 after characters in Terry Pratchett novels, and one after Lady Gaga.

>>NEWSMAKERS

A longtime proponent of the view that U.S. institutions produce too many biomedical Ph.D.s, Tilghman co-chaired a National Institutes of Health (NIH) working group that recommended in June that NIH take steps to curb the growth in trainees and improve working conditions. After taking a year's leave, Tilghman will return to teaching. http://scim.ag/Tilgh_retire

FINDINGS

Bacterial Cell Death Leads To New Art Form

Ridges in biofilms grown on petri dishes have sparked a fundamental discovery in how cells self-assemble into three-dimensional structures. Gürol Süel, a systems biologist at the University of California, San Diego,

wanted to learn more about the coupling of physical and biological factors determining an organism's shape, so he turned to one of the simplest living systems he could think of: the biofilms of the soil bacterium *Bacillus subtilis*, which develop wrinkles on their surfaces. Before the wrinkles form, discrete patches of cells in the biofilm die, laying down the wrinkling pattern, Süel and his colleagues reported online this week in the *Proceedings of the National Academy of Sciences*. Harnessing their new insight that overcrowding seems to lead to cell death, the researchers plated out cells in different densities to form customized biofilms. More than fanciful, the technique may one day prove useful in tissue engineering and in making biofilms that produce useful chemicals. http://scim.ag/_wrinkle

Mouse Saves Its Skin By Shedding It

Known for their stiff hairs, African spiny mice now have a new claim to fame: They can survive losing 60% of the skin off their backs. Their weak skin rips off in the clutches of attackers, enabling the mice to scoot free. Rather than scarring over, the skin quickly grows back, regenerating hair follicles and glands, something most mammals can't do. Developmental biologist Ashley Seifert of the University of Florida

in Gainesville and colleagues compared lab mice skin to spiny mice skin and found the African rodent's skin tears much more readily, possibly because it has larger hair



follicles. Their wounds also heal much quicker, with much less scarring, the researchers reported on 26 September in *Nature*. Only lizards are known to employ a similar response, but in their case, just

the top layer sloughs off. The mice lose their entire skin, baring muscles underneath, so replacing it is a much bigger task. "It's a nice example that shows the maximum capacity of what the mammalian skin can do in terms of healing," says Elly Tanaka, a developmental biologist at the Technical University of Dresden in Germany. http://scim.ag/African_spiny_mouse



Science LIVE

Join us on Thursday, 4 October, at 3 p.m. EDT for a live chat on a hot topic in science. <http://scim.ag/science-live>

CLIMATE CHANGE

Ice-Free Arctic Sea May Be Years, Not Decades, Away

Last week's record-shattering shrinkage of summertime sea ice was yet another reminder that scientists do not understand how global warming is driving the Arctic toward inevitable ice-free summers. Even the latest climate models clearly aren't getting it right, still calling for the Arctic Ocean to retain substantial summer ice well toward the end of the century.

No one believes that anymore. The now-clearly-accelerating decline of summer ice—punctuated by exceptional losses in 2007 and now in 2012—has persuaded everyone that summer Arctic sea ice will be a goner far sooner than the end of the century. So the full knock-on effects of an ice-free Arctic Ocean—from the loss of polar bear habitat to possible increases of weather extremes at mid-latitudes—could be here in many people's lifetimes.

How far wrong the models might be, however, is still very much in dispute. Researchers who take climate model forecasts of ice area as a starting point tend to give summer ice several more decades. "I think 2030 to 2040 is pretty realistic" for an ice-free summer Arctic, says sea ice specialist Julianne Stroeve of the National Snow and Ice Data Center (NSIDC) at the University of Colorado, Boulder.

But researchers who follow the even-faster-plunging *volume* of sea ice are more pessimistic still. "There are a lot of deficiencies in the state-of-the-art [climate] models," says oceanographer Wieslaw Maslowski of the Naval Postgraduate School (NPS) in Monterey, California. If the rapidly declining ice volume is taken to be the better guide, he says, Arctic sea ice could be gone by the end of the decade. So a few more years of watching and waiting could clarify the ice's fate.

Both the area and the volume of sea ice have been waning in recent decades. In area, this summer's low of 3.41 million square kilometers is 18% below the previous record

of 2007—a loss the size of Texas—and 49% below the summer low of 1979, when the satellite record begins. And eyeballing the figure (below), it's easy to see that ice area has been shrinking at an accelerating rate. From 1979 through 2001, ice area was declining 6.5% per decade, according to Stroeve. Since then, it has been falling on average twice as fast.

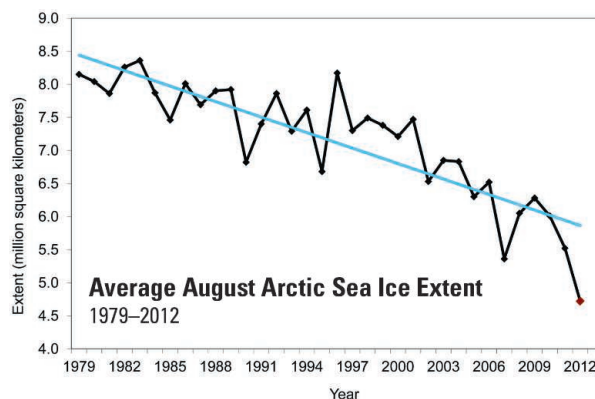
Ice-volume trends are even more alarming. The record of ice thickness is shorter and far spottier than that for ice area, so a group including sea ice specialist Michael Steele of the University of Washington's Applied Physics Laboratory (APL) in Seattle runs a model that calculates the volume of Arctic sea ice. Inputs include measurements of ice area and thickness and observations of atmospheric and oceanic properties such as temperature, cloud cover, and circulation. According to this model, ice volume in August 2012 was 4400 cubic kilometers, 76% lower than in 1979. That's 2.1 standard deviations below the long-term trend, thanks to the more than doubled rate of volume decline during the past decade or so.

Scientists see these trends as increasingly threatening. The less area covered by highly reflective ice, the more sunlight warms Arctic waters, and the faster the ice melts. Rapidly shrinking ice volume denotes another destructive feedback: Ice volume lost through thinning leaves the thinner ice more vulnerable to destruction by rising temperatures and summer storms. "The rules are changing," says sea ice specialist Mark Serreze of NSIDC. Much of "the ice is so thin, it just can't survive the summer," he says.

Going, going, ... This month north of Alaska at 80°N, scientists found sea ice's record-setting edge.

The climate models don't handle changing rules very well, but Serreze takes a shot at a date for an ice-free Arctic Ocean by adjusting the models' projections downward by folding in current trends in ice area and volume. "I'm on record [as of 2007] saying 2030 is a reasonable time for" ice-free conditions, he says. "Ice-free" is generally considered to be less than 1 million square kilometers of ice cover, most of it blown against Canada and Greenland. In that state, "you look at the Arctic from space," Serreze says, "and it's a blue ocean."

Others are looking for a blue Arctic Ocean even sooner. Maslowski and colleagues at NPS run a model simulating Arctic sea ice in more detail than the global climate models do. The model's decline of sea ice volume of late looks much like that from APL's model, but in a paper in the May 2012 issue of *Annual Review of Earth and Planetary Sciences*, Maslowski and colleagues go further and extrapolate recent trends to near-zero volume. Using "only ice extent is not sufficient if you believe volume can change much faster," Maslowski says. Large uncertainties remain, he notes, but their extrapolation gives a date of 2016 for a nearly ice-free Arctic Ocean, with the end likely to come by the end of the decade.



No stopping now. The area of Arctic summer sea ice has been declining rapidly since satellite observations began.

Serreze and others think Maslowski's volume extrapolation exaggerates the problem. "It could happen [by 2016]," Serreze says. "I just don't think so. I think he's being too aggressive." There is, however, a hint that enhanced pessimism may be appropriate. Stroeve is just back from a cruise to 83°N, beyond northern Greenland. She saw only 30% to 40% ice cover there. "We never expected that," she says, because satellite data had not suggested it.

—RICHARD A. KERR

SCIENTIFIC COMMUNITY

U.S. Study Shows Unconscious Gender Bias in Academic Science

Most U.S. professors like to think that they are working hard to overcome the persistent gender imbalance in academic science. They certainly don't consider themselves to be part of the problem. But a new study by a team at Yale University suggests that they're wrong, and that gender bias is deeply rooted in the community.

Last year, a team at Yale University asked 127 professors at six U.S. research universities to judge the merits of a recent college graduate who was hoping to become a lab manager before heading to graduate school. The participants were sent identical resumes, except that half were ostensibly from a female candidate and the other half from a male applicant. The participants—tenured or tenure-track faculty members in the departments of biology, chemistry, and physics—were significantly more likely to hire the man, pay him a higher salary, and see him as more worthy of mentoring. That bias was equally strong among female and male scientists, and did not vary by age, race, or discipline.

Studies of gender bias in science abound, and a multitude of factors, including lifestyle choices, preferences for nonscience disciplines, or even differences in ability, have been offered as possible reasons. This study comes down on the side of “preexisting subtle bias” that is unintended but no less real.

“The debate often gets bogged down in other issues,” says Corinne Moss-Racusin, lead author of the study published by the *Proceedings of the National Academy of Sciences (PNAS)*. “But this eliminates one line of defense. We hope that providing people with inescapable evidence of bias will be the missing piece of the puzzle” in prompting universities to find better ways to address the problem.

Apart from its intrinsic value, understanding the bias within academia is also an essential foundation for any effort to broaden participation in science by women and underrepresented minorities. The stronger the bias, the higher the barrier facing women who want to have a successful career in science. “[T]he current results suggest that subtle gender bias is important to address because it could translate into large, real-world disadvantages in the judgment and treatment of female science

students,” the authors write. They note that it's also the first attempt to directly measure the bias of a representative sample of the U.S. academic community toward the next generation of scientists.

Microbiologist Jo Handelsman, who co-authored the study with psychologist John Dovidio and Moss-Racusin, their postdoc, says that the results shouldn't make faculty



Rethinking science. Diversity is one of the many topics covered in Jo Handelsman's student workshops on “scientific teaching.”

members feel guilty. Rather, they simply highlight the pervasive culture within the community that discounts the value of women. Changing those entrenched beliefs won't be easy, Handelsman adds, but the study provides a place to start.

“The first step is to talk about it, and the fact that unconscious bias can contribute to decisions that we all make,” Handelsman says. “And that's a big step. We're not accusing them of a conscious prejudice or of any venal behavior. But we are saying that they need to be more aware of the effect of this unconscious bias.”

The participant pool was deliberately chosen to mirror the demographics of the academic community. Four out of five respondents were white, with an average age of 50. Three in five were full professors, with the remainder almost evenly split between assistant and associate professors. The applicants were depicted as promising, including having co-authored a journal article with their faculty adviser, but not brilliant, so as not to overshadow the bias being measured.

The team has been working on ways to correct that bias by appealing to the emotional as well as intellectual sides of their academic colleagues at Yale. One project involves local playwrights and artists presenting works that portray successful women and minorities in science. “Art is a good way to reach people emotionally,” Handelsman says. She said that studies have also demonstrated the value of visual priming—showing a positive image of an African American, for example, before judging a pool of job candidates that includes minority candidates—in correcting an unconscious bias.

“Scientists crave data,” she notes. “But when you just give them data, they want to know what it feels like. We'd like to do an experiment that examines whether engaging people's emotions might be more persuasive than just giving them the facts.”

The *PNAS* study was funded by the Howard Hughes Medical Institute (HHMI) in Chevy Chase, Maryland, which for the past decade has supported Handelsman's efforts to improve the quality of undergraduate instruction. “We're not just looking at the learning environment,” Handelsman explains. “If faculty members are treating women differently, they will be disadvantaged not only in the classroom but in every step of their academic training.”

David Asai, who runs the HHMI program, says he thinks the study delivers a sobering message both to organizations like his and to the scientific community as a whole. “Clearly, our efforts to date haven't fixed the problem,” Asai says. “The idea of unintended bias is not new. What is new is their analysis of scientists exhibiting this bias.”

“The study addresses an important issue,” Asai says, “and that's improving the persistence of all students in science. Everybody thinks that someone else is contributing to the problem. But the only way to make science better is if we all own the challenge.”

—JEFFREY MERVIS



ANTHROPOLOGY

Turning From War to Peace In Papua New Guinea

Papua New Guinea has a fearsome reputation for violent conflict. Between 1991 and 2010, about 500 clan wars killed about 1% of the Enga, the nation's largest linguistic group. But on page 1651 of this issue, researchers document an astonishing turn toward peace among the Enga. Starting around 2005, the people began to rely on compensation in village courts to resolve offenses that once might have triggered war, and by 2011, few Enga waged war. "Now people don't want tribal fighting and seem to stop fighting as soon as possible," one former mercenary told the paper's lead author, anthropologist Polly Wiessner of the University of Utah in Salt Lake City, in 2010. "Now nobody comes to hire me."

What caused this turnabout—and can understanding it help calm or prevent warfare elsewhere? Other researchers say the paper offers a rare, fine-grained view of how institutions can promote peace. For example, the data suggest that compensating victims, rather than merely penalizing offenders, may prevent conflict from escalating. "It's a brilliant and important paper," says Harvard University psychologist Steven Pinker, who has studied human violence through time. He praises the study even though it contradicts one of his key conclusions and suggests a higher level of peacemaking in small-scale societies than he has depicted. "Instead of just repeating the commonplace that societies differ in their rates of violence, she has tried to explain *why* they differ, in particular, how they manage conflicts and control their young men," he says.

For more than 25 years, Wiessner has worked with and studied the Enga, highland horticulturalists who depend on sweet potatoes and domestic pigs as staples and are organized into clans of 500 to 1000 people. Wiessner and co-author Nitze Pupu, a blind Enga law school graduate, gathered Enga his-

torical traditions about previous wars, documented more recent ones, and collected records from the village courts, conducting more than 300 interviews.

The switch toward peace came after a plunge into what Wiessner calls a "reign of terror" in the 1990s. Prior to that time, when interclan tensions mounted, personal incidents between members of different clans often triggered warfare. But elders refused to use guns, so wars were conducted with volleys of arrows and were less deadly than most modern wars. Before 1990, "I'd take my lunch and go watch" the war, Wiessner recalls.

All that changed when some young warriors began to use modern, self-loading rifles, and others followed suit in self-defense. War became even more common and much more brutal. Fatalities skyrocketed and schools and villages were burned to the ground. Some armed men became mercenaries or "Rambos," hiring out their services to other clans. One elder recalled to Wiessner that a Rambo told him: "Old man, your time has come and gone." The number of deaths per war—3.7 from 1900 to 1950—leaped to 17.8 between 1991 and 2000. Guns "changed the game completely," Wiessner says. Enga leaders were frustrated and despairing.

But out of this frustration came a change of attitude, starting around 2005, Wiessner says. Elders began to wrest control back from Rambos. War-weary citizens began to turn to local village courts to settle disputes. Rates of violence plunged. The number of deaths per war dropped from 19 in the early 1990s to five between 2006 and 2010,

Pigs for peace. The mood is upbeat at an Enga compensation ceremony, where people distribute parts of pigs to settle inter-clan disputes.

and the number of wars fell precipitously as well. "To date, the relative peace is holding," Wiessner says.

The interviews highlight several factors that helped break the spiral of violence. First, people were tired and impoverished by war and felt that the damage wrought by violence was not worth the potential benefits. Second, once men had eschewed violence, Christianity offered them institutional support for their stance. Third, the brand of justice practiced in the village courts restored good relations among clans.

These village courts, which are distinct from the Papua New Guinea state courts, rarely send anyone to jail. Instead, they direct offenders to pay compensation in the form of pigs and money. Respected local magistrates "weather the rain, the sun, and occasional raucous drunk or pig" to hear cases, Wiessner and Pupu write, and they award compensation, paid by all members of the offenders' clan to members of the victim's clan. (A magistrate might also send parties home to "drink Coca-Cola" together, an adaptation of a traditional sharing of sugar cane.) There is no standard personal punishment for crimes, although sometimes offenders are beaten up, Wiessner says.

Compensation "helps change hearts and minds," Wiessner says. For example, in one egregious case, a man raped and killed a mother of five. Her husband chose to go to village



Here comes the judge. Enga local village magistrates gather after helping to negotiate a cease-fire between clans.

court, where he could be awarded compensation that would help him marry again and pay school fees for his children, rather than send the offender to jail in state court. These data fit well with studies of what's called restorative justice, which focuses on rebuilding relationships and repairing the hurts of a crime. In developed nations, this might involve a medi-

ated conference between offender and victim rather than the gift of pigs, but the principle of trying to help the victim, rather than just punish the offender, holds, says psychologist Michael McCullough of the University of Miami, Coral Gables, in Florida. Studies show that restorative justice soothes feelings of vengeance in ways that conventional criminal justice cannot, he says. "In terms of meeting emotional needs, conventional criminal justice goes begging," he says.

Other researchers emphasize that the paper's detailed look at peacemaking may help illuminate humanity's past, when our ancestors lived in small hunter-gatherer or horticultural societies. Pinker has argued that such ancient societies were quite violent and became more peaceful with the rise of the state. He notes that many of the factors that have pushed humans toward peace throughout the millennia show up among the Enga: the third-party justice of the village courts, a shift in power away from young men, rational analysis of the costs and benefits of war, and religion.

But Wiessner, who agrees with much of Pinker's thesis, differs with him on just how much violence plagued ancestral societies. Even at the height of the gun era, she finds that the Enga had less violence than most small-scale societies in Pinker's analysis. In her view, prestate societies do indeed have mechanisms that can keep the lid on violence. "From having lived in Enga and among the Bushmen, I do not buy the high rates of violence for these societies," she said. "Even a single death sends shock waves through them."

Looking ahead, traditional peacekeeping mechanisms based on kinship may not serve as the Enga transition to a larger society, she says. As people move to the city and clan bonds weaken, clans may not wish to pay compensation for the deeds of their troublemakers. She suggests that times of transition to larger societies may be unusually violent, which may account for the cases Pinker notes. Pinker counters that the village courts are in fact a type of third-party or "state" justice. And he adds that even at their most peaceable, the Enga's rate of death in war is roughly five times that of the United States in the 20th century. But he and others agree that the story of peace among the once-warlike Enga offers insight for the wider world. "The societies that are most experienced at making war," says restorative justice expert John Braithwaite of the Australian National University in Canberra, "often have cultures with the greatest wisdom about how to make peace."

—ELIZABETH CULOTTA



MEDICINE

Drug Trial Offers Uncertain Start in Race to Save Children With Progeria

Parents will do almost anything to save their children, and physician Leslie Gordon is no exception. When her toddler son Sam was diagnosed in 1998 with Hutchinson-Gilford progeria syndrome, she collaborated with noted geneticist Francis Collins to seek the gene responsible for this ultrarare syndrome that seemingly ages children at warp speed. By their early teens, most succumb to heart attacks or strokes. Gordon acquired a Ph.D. and formed a foundation that has allocated millions for progeria research. She has also contributed to many scientific papers about the disease, which she and Collins, now the director of the U.S. National Institutes of Health, argue may provide insight into normal aging. This week, Gordon edges further into new territory. She is the lead author on a publication describing the first clinical trial of a drug for progeria, with Sam, now almost 16, as one of the participants.

More than anything, the trial highlights just how difficult it can be to test a therapy in a terminal disease with so few patients. Twenty-five children, 75% of those known to have the disease worldwide when the trial began, received lonafarnib, a failed cancer drug that lab studies had indicated might help. Gordon and Collins are upbeat about the trial's results—in a press release, Gordon called the results of the lonafarnib trial "a tremendous breakthrough"—but they are in the minority. Most say that the trial design makes it difficult to interpret the drug's effects, or that the treatment simply didn't make a measurable difference. The study was rejected for publication by several top medical journals before finding a home in the *Proceedings of the National Academy of Sciences (PNAS)*.

"It's an experiment in experimenting," says Donald Berry, a biostatistician at the University of Texas MD Anderson Cancer Center in Houston, who has thought long and hard about trials for rare diseases. Berry believes it's critical to try innovative trial designs, especially as molecular medicine stratifies even common diseases into smaller cohorts. But did lonafarnib benefit Sam and the other children? The answer, he believes, is probably not, but some data aren't clear-cut enough to say for sure.

That hasn't prevented Gordon and The Progeria Research Foundation (PRF), which she and her physician husband founded, from moving ahead aggressively long before the results from this initial trial were published. Sam and 44 other children with progeria are currently receiving lonafarnib and two other drugs as part of a follow-up trial started in 2009. And PRF and the researchers it supports are considering whether to add a fourth drug to the mix, one that Collins and colleagues last year showed could reverse some of the cellular defects of progeria.

The condition that Gordon and her family are so desperately battling is caused by a mutation in a gene called *LMNA*, which encodes a protein called prelamin A. In healthy people, prelamin A is altered by an enzyme, farnesyltransferase, that begins its conversion into lamin A, which ultimately helps provide support to the cell nucleus. But in progeria, a mutation truncates prelamin A, this critical conversion to lamin A fails, and patients experience buildup of the abnormal protein in their cells. Among other things, the defective prelamin A distorts the nucleus and alters gene expression. Although they look normal at

Dr. Mom. Leslie Gordon is searching for drugs to save her son Sam from progeria.

birth, toddlers with progeria don't grow properly, and just like elderly people, they begin to lose hair and body fat, and suffer joint stiffness and atherosclerosis. The average age of death is 13.

In 2005, a group led by Stephen Young, a cardiologist at the University of California, Los Angeles, showed that a class of drugs called farnesyltransferase inhibitors (FTIs) seemed to normalize cells from progeria patients. In 2006, his team reported similar success in a mouse model of the disease. As it happened, FTIs had recently been tried in cancer without success, and Schering-Plough (which later merged with Merck), the maker of the FTI lonafarnib, was willing to supply the drug. PRF jumped on Young's mouse work, along with other mouse studies by Collins, and began raising \$2 million for a clinical trial of lonafarnib. The group invited Mark Kieran, a neuro-oncologist at the Dana-Farber Cancer Institute in Boston and Boston Children's Hospital who had been testing the drug in children with brain cancer, to submit a proposal for a progeria trial. Kieran had never seen a child with progeria until he sat with the first family eager to enroll, going over the trial's informed consent form.

Several questions swirled around the study. Would every child get the drug, or would some be offered a placebo to better understand the treatment's effect? And how would improvement be measured? The scientists didn't have enough time or money to track mortality, the ultimate endpoint. They wanted an answer fast.

In preparation for the trial, the foundation had been identifying children with progeria, and collecting and analyzing their medical information in hopes of understanding the natural course of the disease. It was surprisingly difficult to find parameters that could be easily measured and that deteriorated over time in these children. "Many things aren't abnormal," Gordon says. But as she combed through the medical charts of this growing global registry of progeria patients, one thing jumped out at her: Not only did the children "dive right off their growth curve" by age 2, she says, but also the rate at which they gained weight was a straight line, very different from the classic growth curves of healthy children. The foundation sent scales and a logbook to families and for 6 months to a year had them track their child's weight, establishing a blueprint for each individual.

In theory, if a child's weight gain on lonafarnib sent them above the line they'd previ-

ously established, the drug was helping. The strategy also justified the absence of a control group, something Gordon was keen to avoid. Scientifically, control groups are nearly always helpful, researchers agree. Ethically, they can be tricky in situations like this one, where children are dying and there is no treatment. "I don't deny that I would have loved to have a control group," Kieran says. "As long as it wasn't one of my kids that was in" it.

Twenty-five children on lonafarnib were closely tracked for at least 2 years (a 26th died of a stroke during the trial). In the end, nine had a median weight gain of 0.52 kilograms per year, or just over a pound, an improvement over essentially no weight gain the year before. The other 16 either had no significant change in their rate of weight gain or lost weight during the study.

Gordon, Kieran, and their collaborators present this as a modest but encouraging suc-



Genetic finding. In 2003, Francis Collins led one of two teams that found the mutation for progeria, and joined with progeria patient John Tackett, age 15, to announce the discovery. Tackett died the next year.

cess. But Harry Dietz, a pediatric cardiologist at Johns Hopkins University in Baltimore, Maryland, notes that a paper by Gordon and others, published in 2007 in *Pediatrics* and tracing progeria's natural history, reported exactly the same mean weight gain, 0.52 kilograms per year, in untreated children. "I worry that we're interpreting noise," he says, especially given that two-thirds of the participants didn't improve or lost weight while in the trial. A primary side effect of lonafarnib is diarrhea, making it even more difficult to determine whether the drug was effectively combating the disease.

Collins (who did not participate in the study but edited the manuscript for *PNAS*) and Gordon are more excited by a secondary endpoint assessed in 18 children. That measure is carotid-femoral pulse wave velocity, and, in adults, a high reading reflects a

stiffening of the vessel and correlates with heart attacks. No one knows what the measure means in children with progeria—but in the study, 17 of 18 children had a decrease in their pulse wave velocity of between 26% and 48%, indicating that the vessel grew more flexible. "We can change something about the cardiovascular system that has implications for health," Gordon says.

Others are more cautious. "It's a complex paper and very difficult to interpret in my opinion," says Nicolas Lévy, a medical geneticist at the Timone Children's Hospital in Marseille, France, who led a group that identified the progeria gene mutation in 2003. Dietz has hesitated to use pulse wave velocity in his own trials of Marfan syndrome, a genetic disorder that can cause aneurysms, because, he says, it's not clear that modifying it makes children less likely to die.

Another complaint about the trial is that the investigators didn't establish that lonafarnib was modulating its hoped-for target, lamin A metabolism. The only way to test this is by biopsying skin or muscle tissue, Kieran says, and "you can't justify that" in these medically fragile children. Young counters that lamin A metabolism could be measured in white blood cells. Dietz adds: "Especially when sample size is so small, and when you're exhausting a large percentage of the population do to a trial, there should be some very objective measures that the drug you're using is a good drug, and that you're using enough of it."

The lonafarnib trial reported on this week ended in 2010. Gordon, Kieran, and others are now monitoring 45 children from 24 countries participating in the follow-up trial funded by PRF and NIH. They are taking lonafarnib plus the cholesterol drug pravastatin and zoledronate, which prevents fractures. Both pravastatin and zoledronate normalized progeria cells in a dish and had a similar effect when given to mice with a version of the disease. (Lévy and colleagues are currently testing just those two drugs on 12 additional children with progeria.)

Asked whether she worries that her unusual dual role—the mother of a patient, and a driver of new research—leaves her seeing only the silver lining in potential therapies that she hopes will save her son, Gordon says: "It's more important to say yes or no," either a treatment works or it doesn't. "You don't want to delay the process in any way ... with bias. It would just be damaging to the kids."

—JENNIFER COUZIN-FRANKEL

Roots of Empire

A climate history project in Mongolia is charting the unexpected conditions that may have propelled the rise of Genghis Khan

KHORGU, MONGOLIA—As the sun descends over the ancient lava field, John Burkhart—clad in a fluorescent orange field vest, leg guards, and ear covers—sinks a chainsaw into a dead Siberian pine. The saw's earsplitting whine drowns out the hum of insects, until one end of the misshapen log drops to the ground with a soft thud.

As the geography graduate student slices a second time, dendrochronologists Amy Hessl of West Virginia University in Morgantown and Neil Pederson of Columbia University's Lamont-Doherty Earth Observatory in Palisades, New York, watch expectantly from a few meters away. The wood is blackened on one side, probably by a forest fire. That charring has likely protected it from microorganisms and decay, meaning it could have fallen into this crevice between two wedges of volcanic rock hundreds of years ago. The trunk's exposed interior appears free of the rot that would render it scientifically useless. But only after Burkhart extracts a disk of wood a few centimeters thick do the tree-ring scientists celebrate. "That's just an excellent remnant," Hessl says. Pederson grabs a black marker and scribbles a standard field label on the sample, then proclaims, jokingly: "Chinggis looked upon this tree!"

The odds that he is right are vanishingly small. But this chunk of pine could help illuminate the rise of one of history's great leaders. Beginning in the 12th century, Genghis Khan—known in Mongolia as Chinggis Khaan—rose from obscurity to conquer a territory that covered as much as 31 million square kilometers, extending from Korea to the Balkans—the largest contiguous land empire in the history of the world. And yet little is known about how a man of humble origins managed to lead an army that claimed more territory in 25 years than the Romans conquered in 400.

To uncover clues to this puzzle, Pederson and Hessl are spearheading a multidisciplinary project bridging climate science, energetics, and history—part of a growing body of work connecting climate change to centuries-old societal and political shifts. A \$1.4 million grant from the U.S. National Science Foundation, announced last week, will fund fieldwork at Khorgo and other sites over the next 3 years. That research could expand the climate record for a poorly understood region and "develop long, robust chronologies" that "would fill a gap" in climatologists' understanding of medieval Asia, says Ulf Büntgen, a paleoclimatologist at the

Swiss Federal Research Institute for Forest, Snow, and Landscape in Zurich, who is not affiliated with the project.

Samples collected here last year hint at an intriguing history that Pederson says could overturn "the prevailing wisdom on the Mongol empire." Some 8000 to 9000 years ago, an eruption of the now-dormant Khorgo Volcano covered a vast expanse with lava. Today, the landscape is dotted with hawthorn, rose hips, and buckwheat. Siberian pine and larch have a precarious foothold: Some trees protrude awkwardly from cracks in the volcanic rocks, while others are twisted and stunted like bonsai. "This is the edge of what's possible for forests to grow," Hessl says.

Online sciencemag.org

Podcast interview
with Mara
Hvistendahl (http://scim.ag/pod_6102).

That makes the site an ideal barometer for climate conditions on the surrounding grasslands. Throughout the centuries, the lava field's stressed trees have been acutely sensitive to changes in moisture levels. Their rings bear the imprint of those fluctuations.

In 2010, Pederson and Hessl surveyed the area for a project exploring the link between climate change and wildfire risk

Window on the past. Ancient trees that found a foothold in this lava-covered landscape are yielding insights into centuries-past rainfall patterns.

in Mongolia. On their last day of fieldwork, they took samples from 10 living Siberian pines and seven downed logs. They aimed to strengthen a 600-year-long precipitation record derived from lava-field wood by Lamont-Doherty's Gordon Jacoby and colleagues. "We were just hoping for 500 or 600 years" of history, Pederson recalls. In the 13 years he'd been working in Mongolia, the 1500s were as far back as his group of dendrochronologists had gotten.

Their last batch of samples turned back the clock to much earlier. Several trees predated 1300; one was from the mid-600s C.E. A preliminary climate record that Hessl and Pederson constructed from the 17 trees suggests that in the period from 1211 to 1230 C.E., when Genghis Khan was in his heyday, Mongolia enjoyed abundant rainfall: apparently more than in any other 20-year stretch over the past 900 years. The steppe's grasses and other vegetation would have flourished, allowing the Mongols to raise more livestock and giving them what Hessl calls "more horsepower" for conquests.

The tree-ring scientists hope to round out the climate record by uncovering more wood from the time of Genghis Khan and before. They are helped by the fact that many Mongolians venerate the land—some worship trees—and have pointed the scientists to sites strewn with old wood. "Mongolians have really good knowledge about the environment because of our lifestyle of moving around," says project collaborator Baatarbileg Nachin, a dendrochronologist at the National University of Mongolia in Ulaanbaatar. And through sediment analysis and energy modeling, they will attempt to confirm that livestock populations grew as rainfall increased.

For Hessl, an easygoing California native, the project offers a tantalizing opportunity. Research into the relationship between climate and the decline or collapse of civilizations is well-established—from Angkor in Cambodia (*Science*, 20 February 2009, p. 999) to the Maya in Mesoamerica and South America (*Science*, 14 March 2003, p. 1731). But much of that research has focused on water. "When we look back on ancient societies and say lack of water caused an empire to collapse, it's pretty easy to think that won't happen to us. We have reservoirs and other ways of managing water," Hessl says. In Mongolia, her team will look at energy use and availability over the entire arc of an empire, by examining how the Mongols

"captured water and used it to make energy"—for agriculture, for irrigation, for war.

That's a difficult task, notes Büntgen, who led an earlier study that gauged the effect of climate on the rise of the Roman Empire (*Science*, 4 February 2011, p. 578). "With collapse, you have a more precise date," he says, "whereas the rise is vague and less well-documented." But it's one worth tackling, Hessl says: Energy is "a more fundamental linkage and one we can't deny, even in our modern world."

Cryptic conquest

In the mid-1100s, Mongolia's grasslands were an unlikely wellspring for a world conqueror. Nomadic tribes roamed the sparsely populated steppe, frequently waging war with each other. Nor was Genghis Khan an anointed leader. Annals say he was born to a concubine his father had kidnapped. As a young boy, Genghis was banished with his mother and siblings to a bleak corner of the steppe, where he spent much of his childhood scavenging for food. Yet within a few decades, the outcast would unite Mongolia's tribes, and then vanquish peoples across Asia and Europe.

The Mongol conquest "has always been a bit of a mystery," says Nicola Di Cosmo, a historian at the Institute for Advanced Study in Princeton, New Jersey. Chinese troops during the Ming Dynasty sacked Karakorum, the Mongol capital, in 1388, and today only a few artifacts remain. A stone turtle that once marked the city's boundary is now a

lone relic anchoring a grassy knoll. The location of the tomb of Genghis Khan, who died in 1227, is unknown.

Much of what historians know about the ascent of the Mongol Empire is based on a single source: *The Secret History of the Mongols*, an account of Genghis Khan's life written by an anonymous author shortly after the ruler's death. The document disappeared for centuries, only to reemerge in Beijing in the 19th century. Printed in a bedeviling script using Chinese characters to represent Mongol sounds, the text was not fully deciphered until the 1980s. But while rich with details about Genghis Khan's life, the *Secret History* is also heavy on folklore, and it says nothing about resource use or climate during the period of the leader's rise.

Based on the shreds of information that exist, some historians have explained the Mongols' achievements as a product of superior military tactics, proposing that because Genghis Khan's troops grew up hunting and riding horses they had an advantage over sedentary armies. Or, in exploring why the Mongols managed to rule over a large empire, scholars have pointed to an innovative leadership style. Genghis Khan favored meritocracy over cronyism and embraced conquered subjects who pledged loyalty.

In 1934, British historian Arnold J. Toynbee may have been the first to point to a possible climate connection, but he apparently got it wrong. He proposed that a "push exerted by the climate of the steppes"—implicitly an



Tree-ring detectives. Neil Pederson (left) and Amy Hessl are using long-dead Siberian pine and larch (lower right) to reconstruct 13th century climate records.

CREDITS (CLOCKWISE FROM LEFT): KEVIN KRAJICK/EARTH INSTITUTE, COLUMBIA UNIVERSITY; MARA HVIETENDAH (2)

Where Asia's Monsoons Go to Die

KHORGU, MONGOLIA—He has scaled a Bhutanese mountaintop and ventured into Vietnamese highlands, a Costa Rican cloud forest, and the Alaskan tundra in search of ancient tree-ring samples. Few paleoclimatologists are worldlier than Kevin Anchukaitis of the Woods Hole Oceanographic Institution in Massachusetts, yet his first foray into a Mongolian lava field left him awestruck. “This site is pretty exceptional,” he says, bounding over an “ocean of rocks” toward one of the few standing trees on the arid and desolate landscape. After rapping his knuckles on a gnarled Siberian larch—by the sound, he could tell that it wasn’t rotten and hollow—Anchukaitis unsheathes a T-shaped tree corer, screws it into the trunk, and extracts a cylindrical sample about the length and diameter of a pencil.

“Sites in Southeast Asia tend to be wetter and not as moisture-stressed,” he explains. “It’s hard to find any sites like this.” As he eyes the sample’s undulations of dark and light hues, he estimates the tree is about 500 years old. The lava field’s harsh conditions make it a prime location to find slow-growing



Rain man. Kevin Anchukaitis and colleagues are using tree-ring cores (above) to build an atlas that will help scientists model shifts in the Asian monsoon.

inhospitable climate—may have propelled nomadic armies like the Mongols to venture out for resources. “That was not a picture supported by any data,” Di Cosmo cautions. But the idea persisted because of historical records showing a 12th- and 13th century drought in neighboring China and Tibet, and because history was largely written by the people the Mongols conquered. Such an inference is akin to “talking about what happens in Arizona based on what happens in Mississippi and New York,” says Kevin Anchukaitis, a paleoclimatologist at the Woods Hole Ocean-



Unlikely ruler. Raised in poverty, Genghis Khan conquered more territory in 25 years than the Romans did in 400.

ographic Institution in Massachusetts who is assisting on the project.

The tree-ring data collected by Hessler and Pederson show that in the late 12th century, around the time Mongolia was wracked by intertribal warfare, the area did experience a cold, dry period. But a few decades later, as Genghis Khan began consolidating power, weather conditions appear to have substantially improved—and to nomads who rely on access to lakes for watering animals, that would have made all the difference. In times of abundant rain, pastoralists thrive, Hessler says: Very little human effort is needed to “create large amounts of meat that is mobile, that can be used for war, and that can be used to transport things.” Whole herds can be tended by children—leaving the men free to fight.

If more rainfall boosted grassland productivity and overall energy output, that could help explain why the Mongols were able to transition from a “chieftain society, where positions are hereditary” to managing a complex state covering a vast empire, Di Cosmo says: “A centralized state requires more resources.” The horses and food accumulated on the steppe would have enabled the Mongols to set out for China in pursuit of gold and silk—and from there on to more distant lands.

For its scale and grandeur, the expanded Mongol Empire was remarkably short-lived. Climactic shifts may help explain why. The preliminary tree-ring record suggests “a rapid

change that was sustained for a few decades, and then that was it,” Hessler says. Around 1258, after an unidentified volcano unleashed a massive eruption that spewed sulfur and ash into the stratosphere, a cool, dry climate returned. Kublai Khan moved the Mongol capital to Dadu, now Beijing. Thereafter, he ruled as emperor of China, founding the Yuan Dynasty. But outside the region, the Mongols’ power had begun to wane.

Depths of time

After Burkhart sheathes his chainsaw, the researchers hike back to camp with samples from some 30 trees, including larch. Multiple species will allow them to “better capture the climate signal,” Pederson says. They drop their gear in their *gers*, or yurts. Before feasting on lamb goulash and fried fish washed down with salty milk tea, they change into swimsuits as dusk falls and dive into frigid Lake Terkhiin Tsagaan to cool off.

The new samples should help sharpen the climate record, but another piece of the puzzle lies at the bottom of the lake. Starting next year, Avery Shinneman, a postdoctoral scholar in biology at the University of Washington, Seattle, will paddle out into Terkhiin Tsagaan and other lakes to take cores of sediment. The sediment accumulates at the lake bottom like a “layered history book,” she says. Using dating techniques based on the radioactive decay of the isotopes ^{210}Pb and ^{14}C , Shinneman will look for fossil dia-

The Asian monsoon systems shape rainfall patterns over a region home to roughly half the world's population, alternating between relatively dry winters and relatively wet summers. But modeling how a warming climate will impact the monsoons has proved challenging. "When it comes down to creating actual computer models for explaining and predicting monsoon behavior, by and large the models don't yet work that well," says Lamont-Doherty dendrochronologist Edward Cook, who dreamed up the drought atlas. But modelers seeking to forecast future monsoon rhythms can compare their algorithms with the past: "It gives climate modelers grist for the model mill," Cook says.

One driver of the Asian monsoon cycle is the difference between land and ocean temperatures. In spring and summer, land warms more quickly than seawater, causing the winds to reverse direction and blow inland, bringing rain. As the climate warms, this temperature difference will increase, explains Caroline Ummenhofer, a Woods Hole climate modeler who has used records from the drought atlas in her work. "In a warming climate, we would expect enhancement of the land-sea temperature contrast and therefore a strengthening of the monsoon," she says. Another key variable, says David M. Anderson, director of the U.S. National Oceanic and Atmospheric Administration's World Data Center for Paleoclimatology in Boulder, Colorado, is the fact that "a parcel of air can hold more water if the air is warmer." With climate change, Anderson says, "we expect that monsoon rains will be greater; wet areas will get wetter."

Two puzzling questions are how the location of the intertropical convergence zone—the band of rainfall created as a ring of atmosphere con-

verges and rises upward—will shift, and how the role of black carbons in the atmosphere above Asia may impact warming. Nicolas Jourdain, a modeler at the Climate Change Research Centre in Sydney, Australia, has analyzed 60 climate models from leading international institutions in a paper under review at *Climate Dynamics*. Assuming a scenario in which atmospheric CO₂ continues to rise through 2100, Jourdain found that nearly all models that are accurate throughout recent history predict that monsoon rainfall will increase in South Asia during this century, with upticks ranging from 5% to 20%. Yet, he says, there's "no consensus among the models about rainfall" in countries like Indonesia, the Philippines, and Papua New Guinea. "Some models project a rise and others a dip in rains." Varied topography, he adds, makes it tricky "to reproduce the physics of this region."

India's recent experience illustrates just how tricky modeling can be. In recent years, monsoon rains on the subcontinent have weakened rather than strengthened, confusing modelers. This year, precipitation is down 12% across India. One possible explanation, put forward online on 26 June in *Nature Climate Change*, is that black carbon in the atmosphere may be reflecting solar radiation back into space and thus reducing warming.

Mongolia is at the outer edge of the monsoon rain belt, or as Lamont-Doherty dendrochronologist Neil Pederson puts it, "where climate systems go to die." But in mapping the complex climate systems of Asia, past and future, this otherworldly lava field is a vital piece of the puzzle.

—CHRISTINA LARSON

Christina Larson is a writer in Beijing.

toms, or small algae, that can indicate high phosphorus levels in lake water, as well as spores of *Sporormiella*, a fungus that lives in the dung of grazing animals. The idea is to gauge the number of livestock that have frequented the lakes over time. Mongolian lakes are like "gas stations" for thirsty animals, Hessel says—and thus a good way to measure population fluctuations that might be compared with the climate record.

The researchers will use such data to draw conclusions about total energy flows at the time of Genghis Khan. Hanqin Tian, an ecologist at Auburn University in Alabama, will apply a model he developed to simulate how climate change affects grassland productivity, water availability, and energy-use efficiency across Asia. Already, Tian has found that precipitation was the key factor in grassland productivity in Mongolia over the past 100 years. He intends to extend that model back another 900 years.

The team's findings will help fill a "void in the human and environmental history of central Asia," says David Stahle, a dendrochronologist at the University of Arkansas, Fayetteville, who is not involved with the project. From the 11th to the 14th centuries, much of the world was in the grip of the Medieval Climate Anomaly. Europe entered a warm spell, while episodic droughts hit the southwestern United States. Sometime between 1250 and 1350, the global climate turned cooler, ushering in the Little Ice Age.



Ghost town. Little remains of the Mongol capital Karakorum.

Much of the data on the period comes from Europe, leaving open the question of how Asia and other regions fared.

Büntgen hopes the endeavor may even help elucidate the epidemiology of the Black Death, which some scientists believe originated in central Asia. "The extension of the Mongol Empire was probably one factor that allowed the virus to spread," he says.

The research could also provide lessons for coping with modern-day climate change, Hessel says. A decade ago, drought devastated the steppe, prompting a mass migration to Ulaanbaatar. Meanwhile, the burgeoning mining industry is adding to stresses on the water table. Mongol history may prove a stark reminder that while resources can prop up an empire, they are also finite. And

yet, Hessel points out, in the face of apparently severe climate conditions, the Mongol Empire did not collapse. Instead, she says, "they restructured" and diversified energy sources. A revamped Mongol army no longer depended on the health of the grassland.

The tree-ring research is poised to reveal much more about that enigmatic empire. But the team's insights should not detract from the individual pluck behind one of history's great military triumphs, Pederson cautions: Ultimately, "Chinggis did it, and his army did it." Still, he adds, a favorable climate and abundant energy would have helped the ruler consolidate his power. That intriguing possibility could reshape our understanding of the seeds of empire—in Mongolia and beyond.

—MARA HVISTENDAHL

Satellite Labs Extend Science

A new type of lab links Western scientists who want to expand with emerging nations seeking access to world-class researchers

Four years ago, Le Quang Minh and Hoang Zung decided to create a cutting-edge chemistry research center at Vietnam National University (VNU), Ho Chi Minh City. The center would also help train the next generation of basic scientists.

But neither Minh, VNU's vice president of international relations, nor Zung, the director of its science and technology department, could think of a domestic researcher with the scientific heft to lead the center. That's not too surprising, given that one of their goals was to strengthen the research capacity of their home institution. So the two men launched a global search, and on a visit to the University of California, Los Angeles (UCLA), they found someone who seemed to fit the bill.

His name was Omar Yaghi, and his scientific achievements clearly qualified him for the job. The Jordanian-born faculty member is one of the most highly cited chemists in the world and an expert on designing novel porous materials. Yaghi had also shown an ability to work with those from another culture, having formed a mentoring relationship with the International Center for Materials Nanoarchitectonics in Tsukuba, Japan. The icing on the cake was a Vietnamese graduate student working in his lab.

Convinced that Yaghi was their man, Minh and Zung invited him to run the center as a satellite of his own lab. Yaghi readily agreed. "Scientists do science to stimulate their mind," Yaghi says. "I want to go into new territories to explore them."

With Yaghi on board, the three men went to work making the center a reality. The university hosted an international conference to flesh out a research agenda for what they called the Center for Molecular and Nanoarchitecture (MANAR). Then they pitched the idea to the Vietnamese government. After countless meetings, Minh and Zung wrung a promise for \$20 million over 5 years for the center, no small feat in a country with a total budget for science and technology of roughly \$700 million a year.

Work began in 2009 on the lab, to be housed on one floor of a newly built high school. Yaghi's graduate student, Anh Phan, began traveling back and forth to Vietnam in 2-month chunks to oversee construction. MANAR officially opened for business in December.

Yaghi is one of several high-profile researchers who in recent years have opened such satellite labs in other countries. It's a hybrid form of international partnership—smaller, more focused, and less bureaucratic than a formal alliance between two institutions, but broader and more structured than a simple agreement between two like-minded researchers to team up on a project.

Western scientists who have set up satellite labs in other parts of the world say the approach provides a relatively easy way to expand their research group and obtain funding without having to run the peer-review gauntlet in the United States and Europe. In return, the host country buys access to a world-class scientist willing to train its students and strengthen its research capacity. That arrangement typically requires less than 20% of a professor's time, comparable to the amount available to faculty for outside consulting projects. Compensation is also worked out on a case-by-case basis.

Yaghi isn't paid by VNU to direct the center. But VNU has named him a distinguished professor, and the position covers a portion of his travel and administrative costs. Yaghi has also promised to visit as often as needed to keep the research on track and to mentor VNU students. So far he has spent only a few weeks in Vietnam. But e-mail and Skype allow him to stay in close contact with his group of some two dozen students and several senior researchers and professors.

MANAR likely won't be the final satellite lab on his plate. Yaghi is looking into creating labs in several countries in the Middle East, including Qatar, Saudi Arabia, and his native Jordan.

Yaghi, who in January left UCLA to head the Molecular Foundry at Lawrence

Berkeley National Laboratory and join the faculty of the University of California, Berkeley, says he isn't trying to create a global scientific empire. But he would like to help jump-start scientific development in areas that desperately need it.

"These countries are very eager to join the world economy and the world science scene," says Yaghi, who took his satellite arrangements with him when he came to Berkeley. In fact, the three institutions have agreed to back a new center for global mentorship to help other topflight researchers around the world set up their own satellites (see sidebar, p. 1602).

Satellite labs can be a challenge to set up and run, Yaghi and oth-



Global reach. Omar Yaghi (top) with the molecular cages he pioneered, and (above) with Anh Phan (far right) and students at MANAR in Vietnam.

CREDITS (TOP TO BOTTOM): REED HUTCHINSON 2008; COURTESY OF MANAR/TRAN MINH HAI

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ers admit. The mentoring scientist must find local talent to oversee the lab, bridge cultural differences, and learn how to navigate another, unfamiliar layer of bureaucracy. The new lab can crowd out responsibilities at the scientist's home institution. There's the issue of researchers who receive taxpayer-provided funds taking some of their best ideas overseas. And some wonder whether the arrangement is also a good deal for the host country.

A source of ideas

Science has always been an international endeavor, and individual researchers have long formed collaborations that transcend national boundaries.

Among institutions, the most prominent form of cooperation is a direct relationship between two or more universities. Such collaborations, which typically involve an exchange of faculty members and students, not only help to build new research capacity but also aim to improve curriculum and nurture entrepreneurial talent.

But such university-to-university alliances also have drawbacks. They can take years to create and are open only to faculty and students at participating universities. Such large alliances may also generate mismatched partners. Claude Canizares, vice president for research at the Massachusetts Institute of Technology (MIT) in Cambridge, which has an alliance with the National University of Singapore, says MIT has tried to avoid that problem by tapping only those faculty members who are eager to participate and who are already familiar with the work of their counterparts.

The satellite arrangements, which operate on a smaller scale, avoid this hurdle by allowing the new lab leader to set the research agenda and select the team. At the same time, the host country puts up all the research funds. At UCLA, Yaghi pioneered the creation of new porous solids called metal-organic frameworks that are prized as catalysts, among other uses. But his satellite labs are pushing other research topics that his U.S. group doesn't have time to pursue.

The Vietnam center, for example, specializes in making novel porous materials. Its long-term goal is to find applications in the energy sector, such as storing hydrogen and other energy-rich gases, as well as environmental applications, such as designing novel water-filtration systems. In Japan, members of Yaghi's lab are searching for novel met-



New directions. Chad Mirkin, shown here giving a lecture in Singapore, says his satellite lab allows him to expand the types of research he's able to pursue.

als containing polymers. "I want new ideas to challenge me and keep me interested," Yaghi says.

Chad Mirkin, a nanotechnology expert at Northwestern University in Evanston, Illinois, has pursued a similar research strategy at a satellite lab at Nanyang Technological University (NTU) in Singapore that's not part of a larger university alliance. Mirkin's research blends chemistry, materials science, and medicine, and his group at Northwestern is one of the largest in the world, with more than 60 students and post-docs. Even so, his home lab is not large enough to satisfy his ambitious agenda.

He says that NTU has been particularly helpful in translating his group's basic chemistry and materials discoveries into medicine and then carrying out expensive clinical trials. Clinical trials are easier to initiate in Singapore than in the United States, he notes. NTU also pays Mirkin to train between seven and 10 students at a time in Singapore. The group focuses on translational research, such as honing nanoparticle synthesis techniques to control particles' shapes and sizes so that they function better in the body. The group also investigates energy applications of nanotechnology, a priority for the government of Singapore but less important to Mirkin's U.S. operation.

"I've found it to be very productive," Mirkin says. "It's definitely a model that works."

Satellite labs can also serve as a conduit for students to move back and forth between countries. Edward Yeung, an analytical chemist at Iowa State University in Ames, runs a satellite lab of 20 graduate students at Hunan University in Changsha, China. Lehui Xiao, a graduate student at Hunan, was the first to join Yeung's group in China. Last year he transferred to Yeung's group in Ames to finish his Ph.D.

Xiao says he entered graduate school with the goal of getting a job in industry. But working under Yeung hooked him on basic research, he says. Now a post-doc at the University of Washington, Seattle, Xiao says Yeung also "encouraged me to go abroad and broaden my experience."

For his part, Yeung says he's also been energized by running a new lab in another country. "It's like being an assistant professor again," Yeung says.

Running a satellite lab has been a "dream scenario" for Julius Vancso, a polymer scientist at the University of Twente in Enschede, the Netherlands. The lab, at the Institute of Chemical and Engineering Sciences in Singapore, is trying to find materials that will prevent biofouling of ships by barnacles and other unwanted marine organisms. "We have a project manager that runs the administration, reports, and keeps in contact with the funding agency," Vancso says. "I am responsible for the scientific content." Vancso is also excused



GLOBAL RESEARCH UNIVERSITIES

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This is the second article in a series on global research universities. The first (7 September, p. 1162) examined how Asian universities, particularly in Hong Kong and Singapore, are attracting researchers from around the world. This week, we look at a relatively new phenomenon: Western scientists who have created what we're calling satellite labs in other countries. The arrangement allows prominent scientists to expand their research portfolios while the host nation puts up funding aimed at enhancing its research capacity and fostering economic development.

from serving on faculty committees at the institute, an arrangement common to most satellite lab directors.

Vancso visits his Singapore lab quarterly, spending 2 to 3 days listening to research presentations and the rest of the week helping the team write up its results. Then it's off to Holland, or perhaps Germany or the United Kingdom, where he takes part in other collaborations. "This is a very surgical operation," Vancso says.

Striking a balance

Most scientists interviewed for this story spoke glowingly of the benefits of satellite labs. One of the few to raise a red flag when the topic was broached was Timothy Swager, a chemist at MIT.

"Going global is a tricky thing. We live in airports already," Swager says about the lives of most academic researchers. "There clearly have been benefits. But a number of us are worried about the downside. Are we weakening how we operate?" he asks. "There is a concern, when you are pulling faculty away from an institution, that you are diluting the faculty."

In China, some scientists have questioned an institution's decision to pay top dollar to attract high-profile researchers who spend little time in the country. They say such scientists are only there to raise the prestige of the host university (*Science*, 22 September 2006, p. 1721).

Swager does not operate a satellite lab, although he emphasizes that he's not critical of the concept. He also believes that U.S. universities should help other countries looking to improve their science and technology base. "But there has to be a balance," he insists, to ensure that faculty members remain engaged in their



Surgical efficiency. Julius Vancso (left) says short, regular visits to his lab in Singapore help students and lab directors alike to remain focused.

home institutions. Swager also questions how much a host institution benefits from the arrangement. "I don't know how you run a lab when you're only there 2 weeks a year."

Yaghi and others say that success doesn't hinge on face-to-face contact. "We do a lot of stuff by e-mail," Yaghi says, as well as conducting video conferences. Members of his lab in Japan spent five conference calls hashing out the text for one upcoming paper, he notes. "I don't have to be on the ground there [all the time]. But it's nice to go every once in a while."

At the same time, Yaghi agrees that personal contact with students is important and that he's trying to do more than teach research skills. "What you cannot do on video is to show the care you are giving that person," he says. "A good deal of mentoring is a one-to-one activity."

Xiao says that Yeung's extended absences from the Hunan lab weren't a problem. Gaining access to professors at Chinese universities can often be extremely difficult, he says, as most of their time is taken up with teaching and writing proposals. In addition, Xiao says Chinese professors put a priority on helping students find work in industry and play down acquiring research skills. And while Yeung spent far less time in China than his domestic professors, Xiao says, "we got more mentoring from Dr. Yeung than from anyone else."

Still, Yaghi and others readily admit that running a satellite lab comes with a host of challenges. Local politicians may try to influence research agendas, they say, and host-country scientists may resent playing second fiddle to a visiting researcher with more clout.

At the same time, the rules regarding travel and time commitments are usually negotiated directly by the host institution and the

How to Go Global

Omar Yaghi was a teenager in Jordan in 1981 when his father asked him to choose between two starkly different futures: Go to the Soviet Union to become a doctor, on a full scholarship that his father had arranged, or move to the United States and make his own way, with only a few months' support from his family.

His father was offering him the choice of studying in one of the two most technologically advanced countries in the world because Yaghi's prospects for obtaining an advanced education in Jordan were slim. Despite the likelihood of economic hardship if he came to America, Yaghi says the decision was an easy one: "I didn't want anything to do with" the political system in the USSR.

So Yaghi crossed the Atlantic Ocean, found part-time work, enrolled in a community college

in upstate New York, and began learning English. Nearly a decade later, armed with a Ph.D. in chemistry from the University of Illinois, Urbana-Champaign, he launched an academic career that has included stops at Arizona State University, Tempe; the University of Michigan, Ann Arbor; and the University of California, Los Angeles (UCLA). In January, he became head of the Molecular Foundry at Lawrence Berkeley National Laboratory (LBNL) and a professor at the University of California, Berkeley.

The 47-year-old Yaghi says his saga proves that America is truly the land of opportunity. Now he's hoping to offer other aspiring scientists that same opportunity with what he calls the Center for Global Mentoring.

Launched this year, the center is a partnership between Berkeley, LBNL, and UCLA, which is providing \$1.5 million in seed money. Its goal is

to help faculty members at the California institutions and elsewhere emulate what Yaghi has done in setting up satellite labs around the world (see main text, p. 1600).

Yaghi and co-chief of the center David Eisenberg, a proteomics expert at UCLA, already have their eye on their first possible project: a collaboration with Vietnam National University (VNU), Ho Chi Minh City, on cancer research. And it would be built around another immigrant whose scientific career has blossomed in the United States.

Vy Lai grew up outside Saigon, then the capital of South Vietnam. When she was 5, her family joined the flotilla of boat people trying to escape the war between the United States and North Vietnam. Her younger brother died before she and the rest of her family made it to a refugee camp in the Philippines. Eventually, Lai's family settled in Canada, and in 2004, Lai earned a Ph.D. in tumor biol-

group leader. For Yeung, who is officially retired from Iowa State but still advises a handful of students, that means coming to China four to six times a year, for a few days at a time. When National Taiwan University in Taipei asked him to be there 6 months a year for 3 years, he initially balked but eventually agreed to spend between 2 and 4 months there each year. Faculty members with more active research programs typically can't afford to devote this much time to a satellite lab, however, unless the researcher is given a joint appointment.

Under any scenario, however, the head of a satellite lab will need a trusted research partner to run the lab on a daily basis, Yaghi and others says. A homegrown scientist works best. Phan, Yaghi's former graduate student and a Vietnamese native, filled that role at MANAR.

"Every culture has its own way of doing things," Yaghi says. "She coaches me on things unique to Vietnam." After negotiating what he thought were the terms of his research arrangement, for example, Yaghi was surprised when his colleagues in Vietnam came back with a list of additional changes. Phan explained that many Vietnamese think it's rude to disagree openly with people.

But even a native can run into problems managing a satellite lab. Balancing the constantly fluctuating needs for reagents with the country's rigid rules for purchasing and stocking controlled chemicals was a never-ending source of concern for Phan, for example.

Those administrative responsibilities eventually took their toll on Phan, whose husband and young child had remained in the United States. Her grueling travel schedule became untenable after she had a second child last year. So in January, Phan, now a U.S. citizen, took a job with the computer chip giant Intel in Hillsboro, Oregon. Yaghi is still looking in Vietnam for her replacement.

One persistent issue for researchers setting up a satellite lab is how to handle intellectual property (IP). Although each agreement is negotiated separately, Yaghi

says that his arrangement with MANAR is typical: The center retains rights to whatever is developed in Vietnam, while IP developed jointly is split between Yaghi's home institution and the center.

Scientists running satellite labs say they are aware of criticism that their ideas, funded by taxpayers at home, will be used to boost the economies of other countries. But few researchers feel such an argument has merit. "We train [foreign] students all the time who go back to their home countries," says James Heath, a chemist at the California Institute of Technology in Pasadena. Heath recently closed a satellite lab in Singapore after achieving his goal of developing a high-throughput method to generate compounds designed to bind to specific proteins.

Besides, he adds, the economic benefits from a satellite lab are not limited to the host country. Heath's protein capture technology, for example, was spun out to

a start-up company located in southern California to take advantage of the talent and venture capital funding there.

Yaghi believes that the ties created in his satellite labs also give the United States and other Western countries increased access to rising scientific stars. "The pools of talent that made the U.S. great are drying up," he says, referring to reports that many talented students in India and elsewhere are either staying in their home countries for their training or returning home soon after they earn their degree. "This is helping us in a very profound way in keeping our country competitive."

But perhaps the most important benefit of satellite labs is the ability to cultivate friends and allies around the globe. "If we succeed in internationalizing what we do, ... it is a basis for a mutual, respectful relationship based on trust," Yaghi says. "To continue to thrive, we need to engage the world in a mutual, meaningful way. This is a very good way to do it."

—ROBERT F. SERVICE

"We got more mentoring from Dr. Yeung than from anyone else."

—LEHUI XIAO,
UNIVERSITY OF WASHINGTON, SEATTLE

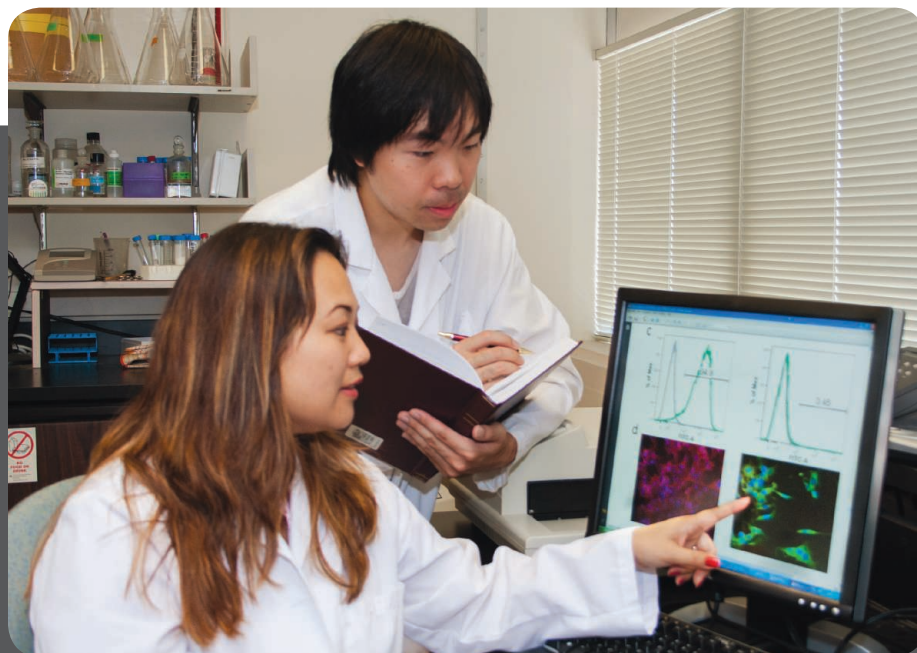
ogy and immunology at the Mayo Clinic's Mayo Medical School in Rochester, Minnesota.

Now a postdoctoral fellow in cancer immunology at the University of Washington, Seattle, Lai is eager to return home. And she is hoping that a cancer center modeled on the materials research satellite lab that Yaghi has created with VNU will give her a chance to fulfill her wish.

"Even though I left Vietnam when I was young, my heart has always been there," Lai says. "My dream would be to go back to Vietnam and help the people there."

For that to happen, VNU must agree to fund the center itself and find outside support for faculty members, students, and research grants. And Yaghi has created a road map for her to follow: "This model has given her a platform to say, 'I can go back to my country.'"

—R.F.S.



Coming home. Vy Lai, with student Matthew Leung, hopes Yaghi's center will help her return to her native Vietnam to launch a cancer immunology lab.



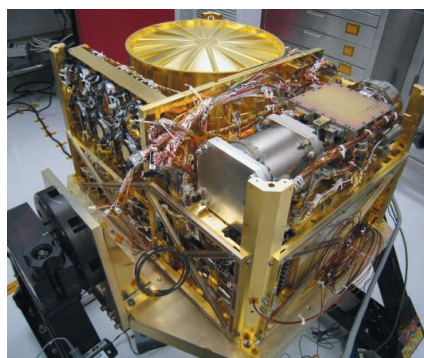
LETTERS

edited by Jennifer Sills

Curiosity and Contamination

R. A. KERR'S NEWS FOCUS STORY ("IN THE HUNT FOR THE RED PLANET'S dirtiest secret," 31 August, p. 1032) covers some of the obstacles that the Sample Analyses at Mars (SAM) instrument on the Curiosity rover faces with respect to the detection of organic compounds on Mars. However, other than a passing comment on detection of chlorinated hydrocarbons by the Viking Gas Chromatograph Mass Spectrometer (GCMS), Kerr does not discuss the critical problem of contamination from spacecraft-borne terrestrial residues.

Given that the SAM organic compound detection limit is in the range of less than one part per billion (1), even minor contamination could affect the analysis results. Any spacecraft component that comes into contact with a sample during the accession procedure before it is processed by SAM could add contaminants. Sources may include resins, wiring, adhesives, lubricants, biologic detritus, and descent rocket exhaust. Curiosity and its components were rigorously cleaned to avoid



The Sample Analyses at Mars instrument.

forward contamination from Earth to Mars, but it is impossible to remove all organic terrestrial residues, and some compounds can be released by degassing and heating during the launch, cruise, and landing stages.

In the case of two Viking landers, in a ~100-mg sample, the Viking-2 GCMS detected several parts per billion of compounds such as Freon E, acetone, benzene, toluene, xylene, and dichloromethane. The Viking 1 GCMS detected only chloromethane (2). These findings were attributed to contamination (2) and not only demonstrated that the GCMS instruments were operative, but also showed the variability in contaminants that might be added to a sample. Recently, three and a half decades after the Viking missions, there have been suggestions that the chloromethane and dichloromethane detected by Viking were in fact derived from perchlorate-based chlorination of indigenous martian organic matter (3), although this claim has been refuted (4).

Contamination issues underscore the potential difficulties and uncertainties in the determination of whether any organic compounds detected by SAM during the Curiosity mission are explicitly of martian origin.

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Religiously Protecting
Myanmar's Environment

THE NEWS FOCUS STORY "AS ISOLATION ENDS, Myanmar faces new ecological risks" (C. Schmidt, 17 August, p. 796) and a recent Letter (1) brought to light the environmental and biodiversity concerns in Myanmar. As the country opens up from decades of isolation, the world's corporations and investors will be eager to enter this new market and exploit its natural resources. Conservationists should recognize the powerful role of religion in Burmese society and engage its potential for supporting sustainable development.

Monks are much revered in Myanmar, where the majority of citizens are Buddhists (2). Other Buddhist countries have shown how the religion can support conservation efforts. In an essay to a conservation journal (3), the 17th Karmapa reflected on the environmental problems faced by Tibet and the rest of the world today. The 14th Dalai Lama was the first to sign a Buddhist declaration on climate change (4). Trees have been "ordained" in northern Thailand in an attempt to prevent logging of the forests in which monks live and meditate (5). Environmental protection is a key mission for the Tzu Chi Foundation (6), one of the largest Buddhist organizations in Taiwan. Buddhism teaches that all things

are interconnected; such an ecological worldview makes for agreeable partnership with conservation scientists (3, 7).

In Myanmar, the Saffron Revolution—led by Buddhist monks—preceded current political reforms. The monastic community is therefore obliged to follow through to mitigate social and ecological ills resulting from development resulting from these reforms.

Working with Buddhist community leaders may not solve some challenges, such as lack of financing for conservation programs or poor legislation. Religion, however, is a powerful moral force that can succeed where science cannot in guiding sociopolitical action and economic behavior to achieve



The shape of the Sun

1611



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conservation goals (8). With such collaboration, Myanmar has a chance to transition smoothly from being developmentally backward but biodiversity-rich to an environmentally sustainable and robust economy.

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U.S. Basic Research: Delayed Drug Development

IN HIS EDITORIAL “NIH BASICS” (3 AUGUST, p. 503), F. S. Collins articulates the need for continued support of basic research by the U.S. National Institutes of Health (NIH) to enable future therapeutic discoveries. We have gathered more information about the relationship between basic research and new drugs.

In 2011, the Food and Drug Administration (FDA) approved 17 new drugs that were considered notable therapeutic advances (1). We reviewed the reference lists from publications about these drugs to determine the most recent basic science paper that contributed to the drug’s development. We defined basic science papers as leading to understanding or description of a biologic process without studying any drug candidate. The publication dates of the most recent basic papers for each of the 15 drugs reviewed (two drugs approved in 2011 were excluded) ranged from 1988 to 2007, with a median of 1996 (2). Eight of the 15 papers were from U.S. labs.

The earliest patent in the FDA Orange Book for each of the 15 drugs averaged 2 years after the most recent basic paper. Inter-

est in developing these drugs may have preceded the most recent basic publications. An outsider cannot know how important these recent papers were in the decision-making for developing these drugs. Despite these caveats, our findings are consistent with other data showing the delay between basic research and applied results. Paul *et al.* cite an average duration of 13.5 years for drug development after a drug target has been fully identified and validated (3). The 16-year median interval from the most recent basic paper to drug approval fits with this duration of specific drug development and illustrates the need to shorten the drug development process. It confirms the importance of basic research now for therapeutic advances in the intermediate as well as the long-term future.

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U.S. Basic Research: Still Number One?

IN HIS EDITORIAL “NIH BASICS” (3 AUGUST, p. 503), F. S. Collins cites several impressive research accomplishments to exemplify the outstanding benefits of support for basic research. It would be valuable to know when these research achievements occurred and what the level of basic research funding was at those times. In addition, a graph of the amount of funding for translational research compared with basic research over the past three decades would illustrate the overall and unusual shift in U.S. National Institutes of Health (NIH) funds.

During the senior Bush presidential era, I had the privilege of testifying to a House committee on NIH and National Science Foundation funding. Former House Speaker Nancy Pelosi asked pointedly whether the United

States was still number one with respect to its basic research position in the world. I answered yes. However, other nations are increasing their basic research funding. If asked the question again today, the rapid movement by NIH to translational funding might require me to change my answer.

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CORRECTIONS AND CLARIFICATIONS

Reports: “UV dosage levels in summer: Increased risk of ozone loss from convectively injected water vapor” by J. G. Anderson *et al.* (17 August, p. 835). The print publication did not note that the paper had previously been published on 26 July 2012 as *Science Express*. The HTML and PDF versions online have been corrected.

TECHNICAL COMMENT ABSTRACTS

Comment on “The Geometric Structure of the Brain Fiber Pathways”

Marco Catani, Istvan Bodi, Flavio Dell’Acqua

Wedeen *et al.* (Reports, 30 March 2012, p. 1628) proposed a geometrical grid pattern in the brain that could help the understanding of the brain’s organization and connectivity. We show that whole-brain fiber crossing quantification does not support their theory. Our results suggest that the grid pattern is most likely an artifact attributable to the limitations of their method.

Full text at www.sciencemag.org/cgi/content/full/337/6102/1605-d

Response to Comment on “The Geometric Structure of the Brain Fiber Pathways”

Van J. Wedeen, Douglas L. Rosene, Ruopeng Wang, Guangping Dai, Farzad Mortazavi, Patric Hagmann, Jon H. Kaas, Wen-Yih I. Tseng

In response to Catani *et al.*, we show that corticospinal pathways adhere via sharp turns to two local grid orientations; that our studies have three times the diffusion resolution of those compared; and that the noted technical concerns, including crossing angles, do not challenge the evidence of mathematically specific geometric structure. Thus, the geometric thesis gives the best account of the available evidence.

Full text at www.sciencemag.org/cgi/content/full/337/6102/1605-e

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

Comment on “The Geometric Structure of the Brain Fiber Pathways”

Marco Catani,^{1,2*} Istvan Bodi,³ Flavio Dell’Acqua^{1,4}

Wedeen *et al.* (Reports, 30 March 2012, p. 1628) proposed a geometrical grid pattern in the brain that could help the understanding of the brain’s organization and connectivity. We show that whole-brain fiber crossing quantification does not support their theory. Our results suggest that the grid pattern is most likely an artifact attributable to the limitations of their method.

In the history of neuroscience, the development of new methods to investigate brain anatomy has been pivotal to our understanding of the complexity of cognition and behavior

(1). Nevertheless, newly developed methods need to be validated and their limitations precisely identified under rigorous experimental conditions before trying to infer general principles of brain

organization derived from their application. Golgi, for example, reported that the brain was organized like a “continuous net” (i.e., reticular theory) because his staining method did not reveal the presence of synapses (2).

Over the past 10 years, advances in diffusion magnetic resonance imaging have opened a new window into the architecture of human brain

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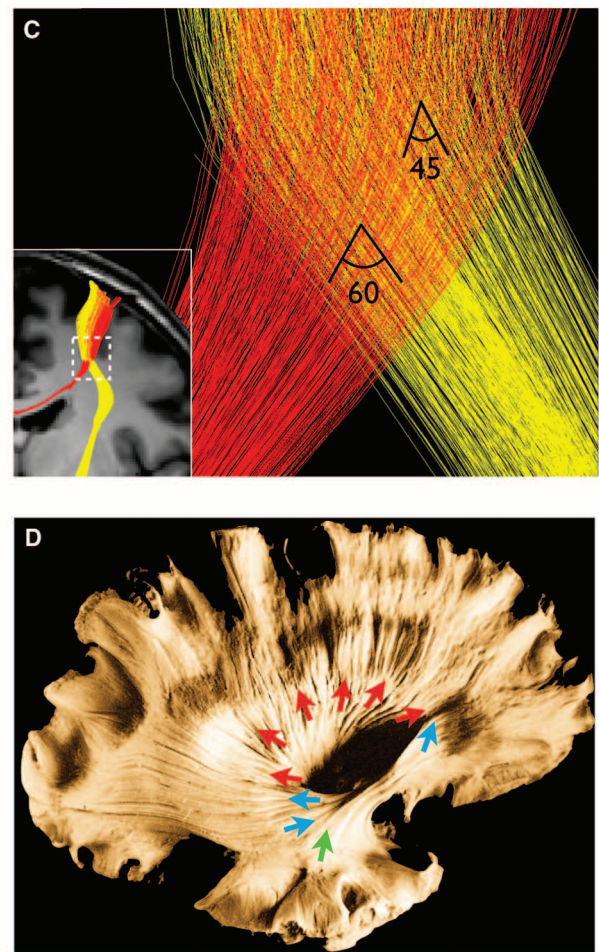
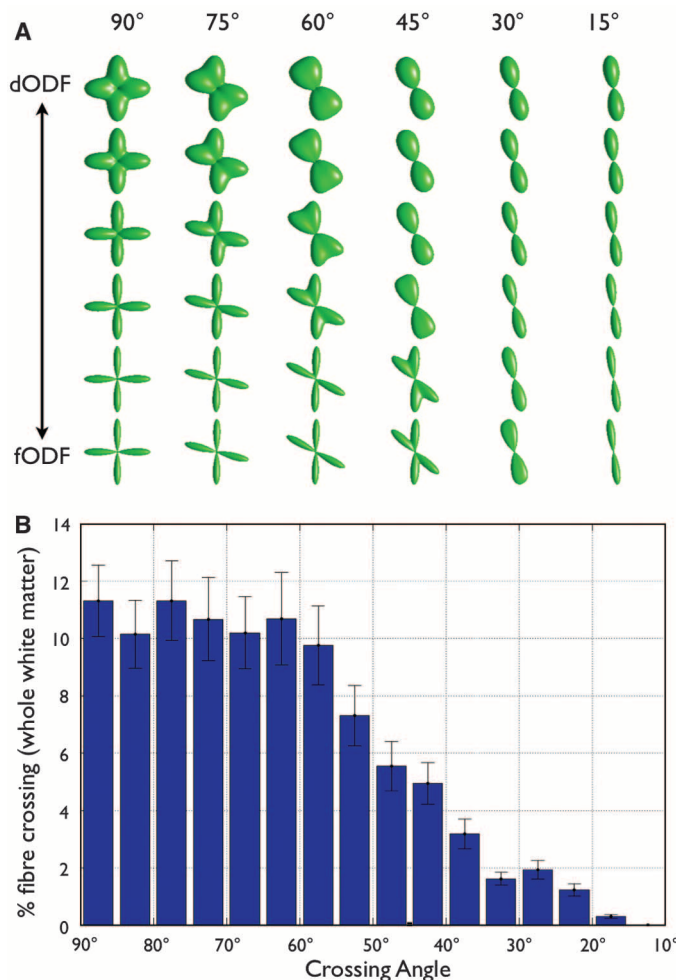


Fig. 1. (A) Visualization of the possible ODF profiles according to different methods and different crossing angles. Some dODF methods are limited in resolving crossing below 75°, whereas fODF methods resolve crossing at 45° or lower. (B) Distribution of the percentage of voxels containing fibers crossing at different angles in a sample of 10 healthy human brains. The plateau between 55° and 90° suggests that orthogonal crossing is not the most prevalent configuration in the human brain. The histogram is likely to underestimate the presence of crossing angles <45°, and therefore the 12% prevalence of 90°

crossing probably represents an overestimation. (C) Tractography reconstruction of the crossing between the corpus callosum (red) and the corticospinal tract (yellow) connections that, according to (3), contains only orthogonal fibers. In this reconstruction, based on SD, the crossing is at angles of 60° or lower. (D) Postmortem blunt dissections of the thalamic projections (red arrows), inferior-fronto-occipital fasciculus (blue arrows), and uncinate (green arrow) show that the three tracts fan out or merge to run in parallel rather than crossing orthogonally [modified from (15)].

networks. Wedeen *et al.* (3) used diffusion spectrum imaging (DSI) to support a theory that white matter fibers form a regular grid by crossing almost orthogonally and uniformly in the entire brain. We believe that this interpretation is open to criticism due to the intrinsic limitations of DSI. DSI resolves multiple fiber orientations by estimating the average diffusion propagator within each voxel and extracting the orientation distribution function (ODF) in which each lobe corresponds to a dominant diffusion orientation. Fiber orientations are then extracted as local maxima of each lobe. Many diffusion methods are available today to resolve multiple fiber orientations. Some methods, such as DSI, qBall imaging (4), or diffusion orientation transform (5) estimate smoother ODF profiles directly from the diffusion characteristic of the fiber (i.e., diffusion-ODF or dODF) (Fig. 1A, top row). Other methods are able to obtain sharper ODF profiles by extracting directly the underlying fiber orientation (i.e., fiber-ODF or fODF) using a specific diffusion model for white matter fibers. The latter approaches are usually described as spherical deconvolution (SD) methods (6, 7), and they generally show a higher angular resolution (i.e., the ability to resolve crossing fibers at smaller angles) compared with methods based on dODFs (8) (Fig. 1A, bottom row).

Figure 1A shows a possible range of solutions for decreasing crossing angles for both dODF and fODF methods. For orthogonal crossing, all ODF profiles are able to distinguish two orientations, whereas for lower angles, only sharper profiles are able to resolve the crossing. Wedeen *et al.* (3) use a method that—according to previous publications where similar acquisition protocols were used and more methodological details were available (9–11)—is likely to have an angular resolution closer to the top row of Fig. 1A. This low angular resolution has a negative impact on the tractography reconstructions shown in (3) because it does not allow separation of fibers that cross at nonorthogonal angles, thus making a grid structure of interwoven sheets a very likely configuration. Additionally, the low angular resolution creates artifactual trajectories, such as streamlines stopping in the deep white matter (3), which is not consistent with the known anatomy.

As an example of the high probability of nonorthogonal crossing in the human brain, Fig. 1B shows the distribution of the angles of fiber

crossing in a sample of 10 healthy human brains based on a SD approach that has been demonstrated to consistently resolve crossing above at least 45° (7). These data show that orthogonal crossing is as likely as nonorthogonal crossing and represents less than 12% of the total crossings in the human white matter. These findings question the hypothesis of a brain consisting of a net made of orthogonal intertwined connections. Furthermore, the experimental results reported by Wedeen *et al.* (3) are mainly qualitative. In our view, the lack of a quantitative and comprehensive analysis of the entire brain across individuals limits their ability to extend their conclusions to the whole brain or infer any interspecies reproducibility. Indeed, our group-based analysis suggests that the analysis performed on selected regions of interest (3) led them to generate a systematic error that replicates across regions of interest and between brain samples of different animal species.

To demonstrate that their method is limited in visualizing pathways crossing at smaller angles, Fig. 1C shows a tractography reconstruction of a region from (3) to contain only orthogonal crossing. In our reconstruction, the crossing of the corticospinal tract with the corpus callosum is mainly at 60° in the lower region and at smaller angles in upper regions (12). This nonorthogonal configuration is common also for many other tracts, such as the uncinate and inferior fronto-occipital fasciculus in the extreme capsule (Fig. 1D) or the splenium and optic radiations in the occipital lobe. Furthermore, crossing is not the only configuration in complex fiber architecture. Fanning, merging, and kissing are other modalities that are frequently observed in postmortem anatomy and not visible with current diffusion methods (Fig. 1D) (13). Finally, the grid model does not take into account the presence of thalamic fibers, which project radially in all brain regions. This implies that most white matter voxels have multiple populations of fibers where, in the same plane, thalamic, callosal, and other projection tracts merge at progressively tangential angles to reach the same cortical areas.

Unfortunately, DSI, as well as other diffusion methods, is limited in resolving these configurations and, therefore, all current tractography reconstructions are biased toward solving only crossing. This information is well known to anatomists, and there is a serious risk in proposing the

grid model as “a means to validate MRI tractography through consistency with grid structure” (3). Finally, current diffusion methods have a relatively low spatial resolution compared with neurohistology. Diffusion-based reconstructions of streamlines should, therefore, not be equated to axonal pathways because they clearly represent an oversimplification of the true white matter complexity inside each voxel.

Diffusion imaging is certainly a promising technique for the study of white matter architecture. Wedeen *et al.* (3) have reported findings suggesting that the human brain is organized like a three-dimensional New York City street grid (14). We conclude that this view is biased by the limits of their technique and does not correspond to the real anatomy. To us, the architecture of the brain, seen through the lens of alternative diffusion methods, bears a closer resemblance to the intricate streets of Victorian London.

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Response to Comment on “The Geometric Structure of the Brain Fiber Pathways”

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In response to Catani *et al.*, we show that corticospinal pathways adhere via sharp turns to two local grid orientations; that our studies have three times the diffusion resolution of those compared; and that the noted technical concerns, including crossing angles, do not challenge the evidence of mathematically specific geometric structure. Thus, the geometric thesis gives the best account of the available evidence.

In a Technical Comment, Catani *et al.* (1) question our findings of geometric structure of the pathways of the brain (2), suggesting that they may be technical artifacts. First, Catani *et al.*'s imaging and analysis of the example of the corticospinal tract is not completely accurate. Classic degeneration studies show that corticospinal pathways make exceedingly sharp turns between local expressions of two cardinal axes, confirming the grid thesis rather than refuting it (3). This feature is not reflected in the image presented by Catani *et al.*, but is reflected in our images, presumably owing to improved technical characteristics *ex vivo*. Second, the account of resolution in diffusion magnetic resonance imaging (MRI) given by Catani *et al.* is incomplete. We show that the resolution for tissue microstructure in our *ex vivo* experiments is approximately threefold higher than the *in vivo* studies compared. Third, Catani *et al.* do not address the central observation of the study—the sheet structure of crossing pathways—or the evidence that this structure is both pervasive and *a priori* exceedingly improbable, hence equally unlikely to be an artifact. Thus, the thesis of geometric structure best accounts for the available evidence.

Catani *et al.* present the corticospinal tract as a counterexample to our model of geometric structure, showing diffusion MRI that depicts

corticospinal and callosal paths as crossing in a continuum of angles from 60° down to 0° angles, identical orientations, at the vertex. Even if we allowed that the 60° crossing was consistent with developmental deformation of cardinal orientations at this location per our thesis, a continuous series of crossings down to 0° would be inconsistent with continuous deformation (2). Specifically, if the callosal paths express the transverse direction of a coordinate system and the projection paths as they appear in Catani *et al.* express the rostrocaudal, then they would need to remain distinct.

More detailed analysis of the corticospinal tract resolves this difficulty consistent with the geometric hypothesis. In a classic degeneration study, Krieg (3) showed that corticospinal fibers have extremely sharp turns; after an initial segment from the cortex parallel to callosal paths, they turn caudally, forming a rostrocaudal orientation field at each point nearly perpendicular to that of the callosal fibers (Fig. 1, A and B). Thus, corticospinal trajectories adhere to the local expressions of the transverse and rostrocaudal cardinal axes down to an exceedingly small scale, one below the resolution limits of these MRI studies. This observation lends our geometric thesis new and independent support and scope. We add that Krieg also finds equivalent structure in humans (3).

Thus, diffusion spectrum imaging (DSI) in the rhesus shows terminations of corticospinal paths where they turn sharply (Fig. 1C). This should be expected with current MRI tractography predicated on orientation continuity (i.e., path curvature $< \sim 1$ rad/voxel) (4, 5). Conversely, the smooth appearance of corticospinal paths shown by Catani *et al.* is consistent with an artifact produced by low spatial and/or diffusion resolution, both of which will tend to smooth out sharp turns. This case indicates that grid structure has implications for the MRI tractography of connectivity (2). Closely related, these sharp turns seriously challenge hypotheses that brain pathways adhere to minimal or geodesic paths

in any metric (4) or are determined by mechanical tension in any simple way (6).

In the present case, the role of resolution in diffusion MRI is to enable the differentiation of populations of fibers within a voxel based on their orientation (4, 5, 7–9). The principal determinants of resolution of microstructure in diffusion MRI are the physical parameters of the encoding, whereas sampling pattern is secondary. Thus, for example, diffusion tensor MRI, however sampled, has little or no ability to resolve crossing (7–10).

We have proposed a model of resolution in diffusion MRI that includes the combined effects of the spatial frequency and mixing time, q and Δ , of the diffusion encoding (11). To map unknown microstructure, the resolution R_{eff} of diffusion MRI may be considered the sum of the encoding resolution $r = 2\pi q^{-1}$ and a spatial blur due to diffusion itself, whose radius is the Einstein length $(2D\Delta)^{1/2}$ for diffusivity D . As these are uncorrelated,

$$R_{\text{eff}} = (r^2 + 2D\Delta)^{1/2} \quad (1)$$

Assuming brain diffusivity $D \geq 0.2 D_{\text{waters}}$, then the *in vivo* studies of Catani *et al.* would have $R_{\text{eff}} \approx 20 \mu\text{m}$ and our *ex vivo* studies with $R_{\text{eff}} \approx 6.5 \mu\text{m}$, or more than threefold improved resolution. As shown in the supporting materials, improved diffusion resolution yields improved detection of crossing fibers (12).

Regarding the choice of DSI over Q-ball imaging or high angular resolution diffusion imaging, these latter methods effectively sample only a subset of the DSI; setting aside noise effects, they are equivalent to DSI with a band-pass filter (8–10). Hence, we believe that DSI should present the lower risk of bias (5) and consider this more conservative choice appropriate when microstructure is uncertain or at issue.

We have claimed that the pathways of the brain adhere to a three-dimensional (3D) coordinate system derived by smooth deformation from the three cardinal axes of development. The clearest evidence for this structure in the mature brain is the sheet structure. Although the body axes in the early embryo were orthogonal, they do not in general remain so in the mature brain owing to plastic deformations of development and growth that lead to curvatures in the lobes, sulci, and gyri in the mature brain structure. This follows from the theorem that the only conformal, or angle-preserving, mappings in three dimensions are rigid motions or isotropic scaling. Such deviations from orthogonality were qualitatively observed in all species [figure 4 in (2)] and measured in rhesus, where they were found to increase from cerebral center to mantle to surface [figure S2D in (2)], consistent with known characteristics of developmental deformation (13, 14). We fully agree that non-right-angle crossings exist, but this is consistent with and indeed required by our thesis that brain

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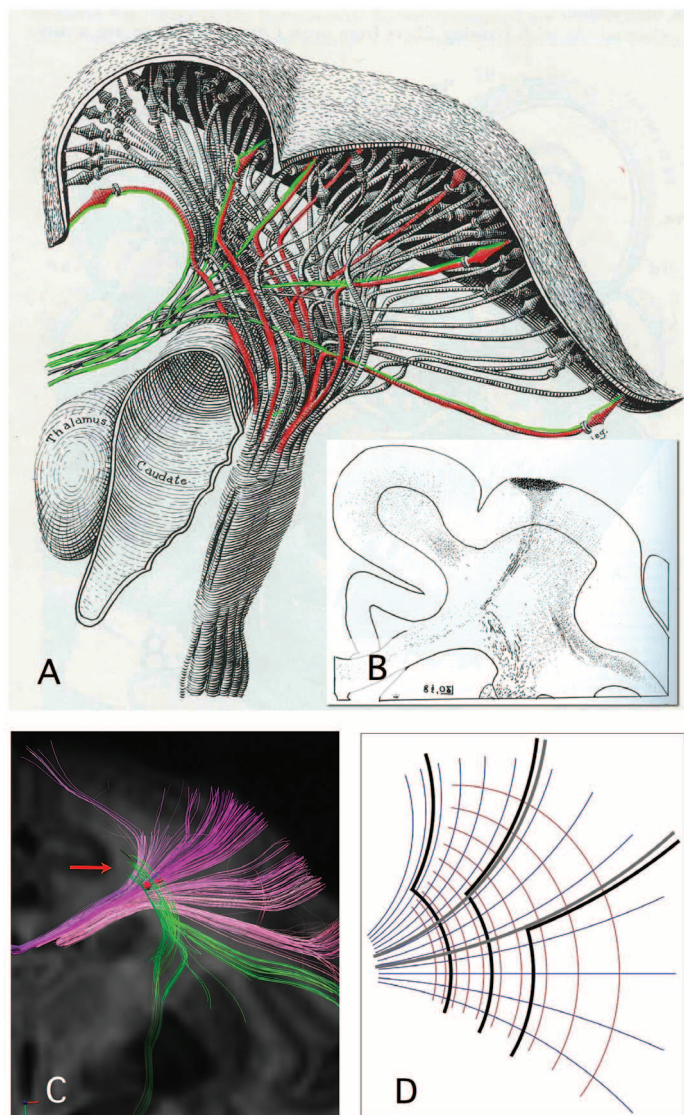


Fig. 1. Corticospinal and callosal pathways of the rhesus monkey, modified from W. J. S. Krieg, drawing (A) [(3), figure 37] and degeneration study (B) [(3), figure 96], with permission. In (A), corticospinal paths (red) leave the prefrontal cortex parallel to transverse callosal paths (green), then turn sharply by approximately 90° (“drop”) to the caudal direction. Even medial corticospinal paths from the vertex show this turn as small as sharp kinks. The degeneration study shown in (B) shows an example of primary data upon which (A) was based. Here, the path turns are so sharp that they show no visible curvature at the submillimeter resolution of this optical tracing. In DSI of rhesus monkey ex vivo (C), rostrocaudal segments of corticospinal paths (vertical) are nearly perpendicular locally to curved callosal paths (horizontal). These rostrocaudal segments terminate among transverse paths (arrow), consistent with turns too sharp to resolve in this study. An illustrative model of the geometric thesis [the mapping $f(z) = 1/(z + \sqrt{-1}) + 1/(z - \sqrt{-1})$ of the complex variable z] (D). The callosal paths (gray) adhere to a transverse coordinate (blue-gray), whereas corticospinal paths (black) adhere to both transverse and rostrocaudal (red) orientations via sharp 90° turns.

pathways adhere to local expressions of curved cardinal coordinates.

Central to our thesis is the finding of sheet structure in cerebral fibers. We have shown that the pathways of the brain are equivalent to coordinate functions because they form in crossing parallel

2D sheets that fill 3D space like pages of a book. As we emphasize, this is mathematically specific and highly atypical, entailing long-range correlations between paths that are as nonrandom as a lock and key (having prior probability ≈ 0). This property does not depend on fiber orthogonality or the

absence thereof—the concern of Catani *et al.*—but rather on a 3D relationship among crossing planes at different locations (the Frobenius integrability condition). As we have shown, this can be represented as an angle between subsheets of fibers, which must be as close to zero as noise allows [figure S2C in (2)], or by the topology of the embedding of the reconstructed paths in 3D, which must be interwoven rather than mutually helical [figure S2A in (2)]. Whereas this sheet structure may be obscured by low resolution or other technical limitations, no mechanisms are known whereby these limitations will create it as an artifact. Because we have observed this structure to be pervasive in cerebral white matter, homologous across species, consistent with embryogenesis, and consistent across diffusion contrast mechanisms, with and without Fourier-Radon reconstruction, we conclude that this organization is real and characteristic of the brain pathways (2). The thesis that brain pathways adhere to a simple geometric system best accounts for the available evidence—not like London, but Manhattan; not unfathomable, but unlimited.

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Supporting Materials

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Supporting Text

Fig. S1

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GEOSCIENCES

A Coevolutionary Tale

A. D. Anbar

Young geologists learn early that ours is an unusual science. In chemistry, physics, and biology, the objects of study and the forces that affect them are usually close at hand. With effort, they can be isolated, analyzed, and experimented upon. Geologists, seeking to understand how Earth came to its present state, face a different challenge. Our object of study is difficult to isolate, and the forces that shaped it are inaccessible in time. As often as not, experimentation is impossible. So, we must piece together scraps of evidence to develop the most plausible story of Earth's past.

In *The Story of Earth*, Robert Hazen uses his considerable talent as a writer to present the latest version of this story. A mineralogist who has worked at some of the world's premier research institutes, Hazen has a unique vantage point. He uses it well, telling a sweeping, rip-roaring yarn of immense scope, from the birth of the elements in stars to meditations on the future habitability of our world.

Anyone new to Earth history will find Hazen's account a revelation. Those who already know that history may nevertheless be entertained by Hazen's telling of it. The

writing is lively and vivid, particularly his imagined hellscape of Earth's earliest years. The chapters are peppered with personal anecdotes and candid opinions. Some are especially colorful, such as Hazen's recounting of the

conflicts between origin-of-life pioneer Stanley Miller and others (including Hazen) over the finding that reactions in deep-sea volcanic vents can produce the building blocks of life. Here and elsewhere, the book will be eye-opening for those used to dispassionate and polite depictions of science and entertaining for others. A good brawl often makes for compelling reading.

But Hazen aims higher than storytelling and entertainment. He wants to convey a



A sign of the start. Hazen sees the microbial oxidation of iron that produced the massive Archean banded iron formations (such as this polished sample from Australia) as the beginning of “the astonishing coevolution of the geosphere and biosphere.”

grand vision of the “coevolution” of life and the inorganic Earth over the eons. Although this is not a “radical new approach” as promised on the dust jacket—in recent times, it was most prominently embodied in the work of James Lovelock (*1*)—Hazen's mineral-centered perspective is fresh. As first outlined in Hazen and colleagues' 2008 review paper “Mineral evolution” (*2*), it centers on the importance of mineral surfaces for the origin and early evolution of life and the effects of an evolving biosphere on the origin and diversity of minerals. The evolution of O_2 -producing photosynthesis and the subsequent “Great Oxidation Event” about halfway through Earth history may have been particularly influential. Most of the minerals on Earth today, Hazen argues, exist because life caused the pervasive oxygenation of Earth's surface environment. He recasts mineralogy as a historical science that is central to understanding life's history on Earth.

Despite these virtues, *The Story of Earth* is not quite the book it might have been. General readers should be a bit wary because in some places the book contains language that can lead to misconceptions. For example, Hazen says that volcanic hotspots derive from rising “plumes of hot magma from the core-mantle boundary,” when in fact the melting that forms magma occurs near the surface. He writes that the enzyme RuBisCO (ribulose-1,5-bisphosphate carboxylase-oxygen-

ase) “absorbs the Sun's energy” as though it contains chlorophyll, when this enzyme is actually part of the light-independent Calvin cycle that uses ADP (adenosine diphosphate) and NADH (the reduced form of nicotinamide adenine dinucleotide) derived from the Sun's energy. And when he states that “all the

types of chemical bonds introduced so far ... are called ionic bonds,” this apparently includes the silicon-oxygen bonds introduced two pages earlier (which most introductory chemistry and mineralogy students learn are covalent bonds). Granted, it is hard to be precise when writing engagingly for a lay audience. Still, such misconceptions could have been avoided.

Fellow scientists should also be wary of details. For example, when telling the tale of the Great Oxidation Event, Hazen's discussion of organic biomarker evidence confuses hopanes with hopanols. (The former are found in rocks; the latter are their biochemical precursors.)

And he states that the discovery of hopanes in 2.7-billion-year-old rocks is evidence of eukaryotes, when in fact this evidence comes from the discovery of steranes. Further, he does not present the conventional argument that the presence of steranes means that O_2 appeared long before the Great Oxidation Event, because as far as we know the biosynthesis of their steroid precursors requires O_2 . This omission is striking because Hazen makes a point of saying that other evidence of early O_2 , discovered in the 2.5-billion-year-old Mt. McRae shale by this reviewer and others (*3, 4*), is challenged by the concepts in his 2008 review. Why is this a conflict, and why is it isolated to the Mt. McRae study? The explanation provided in the book is inadequate. Sophisticated readers will long for footnotes or chapter notes to help them dig deeper.

None of these are fatal flaws, however. Hazen is a master storyteller with a great story to tell, and so I recommend *The Story of Earth*. But as with any storyteller's tale, read it for inspiration but not as the final, authoritative word.

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The Story of Earth

The First 4.5 Billion Years, from Stardust to Living Planet

by Robert M. Hazen

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PHYSIOLOGICAL ECOLOGY

Far-Reaching Impacts of Energetics

Kimberly Hammond

Animal biologists have always been fascinated with where animals live and why they are often restricted to specific places and climates. As long ago as the 18th century, the invention of the calorimeter allowed biologists to start measuring the metabolic rates of animals to understand the influence of energetics on animal

distribution and life history. In a foundational 1932 paper (1), Max Kleiber reported that across a wide range of mammalian species, metabolic rate (B) scales nonlinearly with body mass (m) according to a

power law, $B = a m^b$. His “mouse to elephant curve” for basal metabolic rates suggested $b \approx 0.75$. The paper spawned work aimed at better determining the value of b (with results ranging from 0.67 to 0.75) and ultimately controversies over the universality and importance of that exponent. [See, for example, (2) and the additional research stimulated by it.] In other extensions from Kleiber’s work, the fundamental underpinnings that determine metabolic energy expenditure, basal metabolic rate, and their influences on animal life history have been the object of intense study over the past 80 years by many different investigators in the field and in the laboratory.

While the overarching questions and conflicting theories have kept the field lively, increasing our understanding of ecological energetics requires empirical data. Among those who have contributed this data, probably no one has made more measurements of metabolic energy expenditure of mammals and birds across a wide range of environmental circumstances than has Brian McNab. Over a career that now spans more than 60 years, he has gained a keen and extensive knowledge of and intuition for the influence of natural history on metabolic relations in endothermic vertebrates.

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In *Extreme Measures*, McNab (University of Florida) draws on and synthesizes his experiences in an attempt to make some sense of the huge diversity we see in endothermic metabolic rates and lifestyles. He surveys the diversity of energetic strategies in birds and mammals from his own perspective, examines some alternative views, and offers an analysis of the often elusive, fundamental underpinnings that he believes determine metabolic energy expenditure. Although the book’s subtitle highlights energetics, through a very large proportion of the book McNab specifically focuses on the influences of basal metabolic rates.

McNab begins with three chapters devoted to fundamentals. In these, he describes the links among environmental temperature, body temperature, body mass, and metabolic rates; explains various methods used to study these rates; and sketches some of the controversies that have arisen over the quantitative analysis of the data gathered through these methods. Although it is clear that McNab finds many phylogenetic contrast analyses circular in their reasoning, he also concedes that the historical context is continually present and shaping species’ lifestyles. Likewise, throughout the book he returns to the influence of phylogenetic history and the constraints that result over and over again.

McNab devotes much of the remainder of the book to an extensive look at birds and mammals in many different habitats and under a variety of conditions. He explores how their ecology and behavior are influenced by the interactions between energetics and such factors as very small and very large body masses, food habits, the hottest and coldest climates, and very dry and very moist environments. He also considers links between energetics and aspects of reproduction, population dynamics, and evolution.

Much of the work the author cites is his own, although he also draws on an extensive literature to support his coverage of many different species. His exhaustive synthesis is to be applauded, but occasionally readers may lose focus while wading through all of the details. In some instances, McNab revisits past controversies with a reanalysis of the questions. Such reevaluations may sometimes seem less than objective, but, after all, part of the reason he wrote the book was to provide his own interpretations of the data—and he has earned that right. Offering enjoy-

able diversions from the data and analyses are the boxes of text scattered throughout the book. These describe McNab’s research adventures (and misadventures) and exotic animals that he was fortunate enough to work with or, occasionally, provide additional technical information or more-detailed explanations.

In his final chapter, McNab recounts the (now very familiar) details of our current condition under the looming specters of pollution, overpopulation, and global climate change. His rather bleak outlook on our current trajectory may leave readers feeling hopeless about avoiding an environmentally induced collapse of human societies. In fact, he suggests that there is little for concerned biologists to do but “study the living fauna exhaustively before it goes.” The concluding paragraph hints that he looks forward to a world without us, as “then the evolution of



Intermittent endotherm. Torpor allows the shrew *Suncus etruscus* (2.2 g) to avoid the energy expenditures required by continuous endothermy at very small body masses.

endothermic diversity can start again.” There is no doubt that humans have placed much of Earth’s biota in deep peril, but I think we ought to attempt creative and positive approaches to undoing some of the troubles we have caused before we consign ourselves to the dustbin.

In *Extreme Measures*, McNab synthesizes data he gathered by studying an incredible range of birds and mammals in diverse environments scattered all over the globe. His all-too-fatalistic position at the book’s end will not help us encourage students to take up science to address the questions and concerns he raises.

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When Scientific Research and Legal Practice Collide

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Legal equality safeguards deliberative process while improving transparency in scientific research.

The modern historical record and an ongoing dispute between BP and academic researchers reveal that the U.S. legal system can be exploited to attack scientific research and academic thought when it challenges entrenched interests or beliefs. These legal practices erode the ability of scientific research and academics to function properly.

In 1954 an economist, Paul Sweezy, was summoned for questioning about “subversive persons” within the United States. He refused to answer questions related to his academic lectures and publications, because they violated his constitutional right to freedom of expression. Sweezy was found in contempt of court and incarcerated. The U.S. Supreme Court overturned this decision in 1957 (*1*).

In 1980, Dow Chemical Company subpoenaed confidential documents from an ongoing study of the carcinogenic potential of defoliants known as Agent Orange from researchers at the University of Wisconsin. Dow sought to use preliminary results from the documents to support its claim that the scientific analysis was inconclusive in a separate legal action against the U.S. Environmental Protection Agency. The researchers were not parties to the case between Dow and the government and refused to comply with the subpoena. The Federal District Court sided with the university researchers [(2), paragraph 5, p. 6], writing that

...In the early stages of any research project there are likely to be false leads or problems which will be resolved in the course of the study with no ultimate adverse effect on the validity of the study. To force production of all information demanded by the subpoenas is likely to jeopardize the study by exposing it to the criticism of those whose interests it may ultimately adversely affect, before there has been an opportunity for the researchers themselves to make sure the study is the result of their best efforts.

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22 April 2010, Deepwater Horizon. This oil platform exploded in the Gulf of Mexico, killing 11 people and causing the largest accidental oil spill in history.

A decade after the Agent Orange decision, a group of tobacco companies subpoenaed the Mount Sinai Medical School for data and documents from two published lung cancer studies. The medical school contested these subpoenas, but the Federal Court sided with the tobacco companies, stating that once the results were published, the public had an interest in resolving disputes that involved the accuracy of the conclusions (3). Subsequent research uncovered systematic attempts by tobacco companies to impeach scientific findings linking health hazards to tobacco use (4–9).

In 2011, as the defendant in a product liability lawsuit, Bayer pharmaceuticals sought confidential peer-reviewer comments from a published study. The Federal Court denied Bayer's request, citing the importance of peer review to “ensure integrity and reliability in scientific activity and reporting,” stating that the “pillars of a successful review process are confidentiality and anonymity; anything less discourages candid discussion and weakens the process” and that “damage to the peer review process can also undermine efforts to improve public health and safety” (10).

Research into the Deepwater Horizon disaster has led to similar legal confrontation. In late April 2010, BP contacted us, a group of academic researchers at the Woods Hole Oceanographic Institution (WHOI), requesting technical expertise to assess the failed blowout preventer. We felt an ethical obligation to assist with the disaster response. We quickly provided recommendations to BP and assembled an on-site operations team to acoustically measure the well's flow rate and to determine the actuation states of the blowout preventer's shear rams. On 6 May 2010, BP preemptively canceled the WHOI assessment operation (11) in favor of an ultimately unsuccessful attempt to deploy a containment dome (12). On 23 May 2010, the U.S. Coast Guard contacted WHOI because “delays in measurement of the oil flow are no longer acceptable and require urgent response” (13). The Coast Guard tasked us with measuring the flow rate and delivering an independent findings report within 5 days of completing operations.

We completed acoustic measurement of the flow rate but were denied by BP the opportunity to collect fluids released from

the well (14), so our preliminary estimate only described total volumetric flow rate (15). We later returned to the site and collected samples (14). For more than a year after this, we, along with our colleagues at other academic institutions, provided assistance at no cost to BP or the U.S. government to develop a theoretically rigorous model of the well fluid chemistry and flow rate. This culminated in two peer-reviewed papers in 2011 (16, 17).

In late 2011, BP subpoenaed WHOI, demanding that “any transmission or exchange of any information, whether orally or in writing, including without limitation any conversation or discussion” be surrendered to BP (18). BP claimed that it needed “to advance BP’s and other parties’ understanding of scientific work” (19) but also made public allegations against us that it portrayed as “not simply one of impeachment, but one of integrity, reliability, and reproducibility” (20).

As is common in scientific research, our analysis evolved, gaining precision over time. Our initial calculations drew from a small subset of data and used simplifying approximations to enable quick estimation under a tight deadline. Intermediate calculations benefited from chemical composition and final calculations from key information about source geometry. All of the earlier estimates are within the error budget of the final peer-reviewed calculation, which indicates that any quantitative differences are statistically insignificant. All of these calculations are also within the range of values that BP’s own engineering staff calculated and then allegedly attempted to destroy (21). Furthermore, these estimates are also within the range of published values from multiple independent studies using separate methods of analysis (22–25). Most important, we promptly supplied BP with over 52,000 pages of materials, including all the information needed to verify our findings (26).

WHOI fought BP’s motion to compel production of additional deliberative and confidential materials because doing so would compromise independent scientific inquiry. The Court recognized the principle that we asserted, writing in its decision that production of these deliberative documents “could hamper future research efforts”; however, the Court still ordered WHOI to surrender these documents to BP “even if the only purpose for the analysis documents is impeachment” (27).

Through other legal actions, BP obtained a prepublication manuscript submission (20) from the editor for one of the WHOI papers (17). Through the subpoena, BP has also

obtained copies of the reviewers’ comments and subsequent confidential deliberations among the authors and peer reviewers.

Like the Dow Chemical case, BP sought prepublication materials from academic researchers who were not party to a court case in order to assert that the researchers’ findings were contradictory or inconclusive. Like the Bayer product liability case, BP sought access to peer-review deliberations with the goal of limiting its financial liability (20). Similar to the tobacco company cases, BP is alleged to have suppressed its own findings (21) while attempting to publicly discredit similar findings in peer-reviewed literature.

Perhaps the most disturbing parallel is to the case of *Sweezy*, because BP has informed WHOI of its intention to depose our team of researchers for questioning with the same jeopardy of sanctions that *Sweezy* faced. Because we are not litigants to the case, having chosen to remain independent and impartial (i.e., not serve as expert witnesses), we have no legal standing in court. Thus, although BP has made generalized assertions of misconduct in the Courts’ public record, our opportunity to challenge these claims is restricted. The law paradoxically requires that, as nonlitigants, we must be found in contempt of court before we are granted legal standing (upon appeal) to defend our work and reputations.

Although it is tempting for researchers to seek the protections used by journalists for confidential sources and material, journalistic privilege claims are tenuous. Unlike attorney-client and doctor-patient privilege, federal legislation for journalistic privilege is absent.

Our academic, governmental, and industrial research communities should work together to better align American legal practice with the deliberative process of science. This can be accomplished through new federal legislation that protects researchers from legal harassment by interests seeking to silence scientific inquiry or retribution for publishing independent research findings.

Legal equality facilitates scientific transparency and accountability. If independent researchers are drawn into a legal proceeding and forced to surrender confidential deliberative documents or submit to deposition, they should be granted the same legal rights that are afforded to litigants. At a minimum, independent researchers should be granted legal standing to defend their work and reputations with the same level of access to legal discovery as litigants. This should include reciprocal subpoena power to examine confidential information held by litigants, as well as the right to seek direct legal remedy for unsubstantiated allegations. Furthermore, if

a litigant seeks confidential documents from peer reviewers and editors, there should be a court-adjudicated finding of fact, requiring compelling evidence of misconduct before these materials are surrendered.

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EVOLUTION

Suppression of Sleep for Mating

Sleep can be seen as an environmentally determined, adaptive behavior.

Jerome M. Siegel

Sleep has been viewed as a maladaptive behavior because it is incompatible with activity required to acquire food, defend against predation, and mate. Yet it appears to be nearly (1) universal among birds and mammals, leading to the assumption that sleep serves an unknown but vital physiological function. However, no function that can explain the huge variation in sleep times within and between species has yet been firmly identified, although many candidates, including reversal of oxidative stress, memory consolidation (2), extension of life span, and removal of various neurotoxins, have been proposed. On page 1654 of this issue, Lesku *et al.* (3) show that in one species of bird, those that sleep the least gain an advantage—they produce the most offspring.

Male polygynous pectoral sandpipers engage in complex courtship displays and aggressive defense of potential mates over 3-week periods. Lesku *et al.* observed that during this time, the males show no reduction in activity or degradation of performance despite little or no sleep. This finding and other recent work undermine the dominant paradigm that postulates a vital physiological function for sleep in birds and mammals. Killer whale and dolphin mothers and their calves are continuously active with eyes open for 6 or more weeks after birth. No rebound of inactive behavior follows (4). During this period, the neonates' brain and body grow to their prodigious size and capacity without any apparent need for sleep-linked detoxification. Adult dolphins working for reward can accurately discriminate between visual stimuli presented at 30-s intervals on their left or right sides, 24 hours per day, for as long as

5 days. During this time, their performance shows no progressive decline. No rebound of inactivity follows the session (5). By contrast, humans whose sleep is interrupted on a similar schedule are dramatically impaired (6), demonstrating the variability of sleep regulation across species. Migrating birds greatly reduce sleep time with intact learning abilities and high rates of performance, with no subsequent sleep rebound (7).

If male sandpipers that are continuously active during the breeding season clearly leave more offspring than males who sleep, why hasn't natural selection eliminated the sleepier males? An attractive explanation is that the active males are more likely to

deplete caloric reserves. Under optimal conditions, this depletion can be prevented by eating more. However, in conditions of food scarcity, the birds that most greatly depleted caloric reserves during mating will be least likely to survive to the next mating season. Thus, in the long run, a dynamic balance should be achieved across a range of sleep times (8, 9).

A similar balance appears to exist in humans. People of similar age, sex, and body build can have very different sleep times. They can also vary in their response to sleep loss, with some being highly impaired, unable to resist sleep, and others showing high levels of

functioning despite sleep loss. The effect of sleep deprivation on performance is not strongly related to baseline sleep duration (10). Furthermore, human sleep duration is not linearly related to health, with both high and low values being linked to shortened life span (11). Death of rats due to sleep deprivation may be related to stress rather than sleep loss. Sleep deprivation has not been reported to cause death in pigeons, mice, or in rats deprived by techniques that do not involve waking them frequently at sleep onset. Fatal familial insomnia can cause death in humans, but sleep loss does not appear to be responsible. This disease affects many body organs (8, 12).

	More sleep	Less sleep
	Golden mantled ground squirrel <i>Spermophilus lateralis</i>	Degu <i>Octodon degu</i>
Total sleep	15.9	7.7
REM sleep	3.0	0.9
Brain/body ratio	0.016	0.010
Life span	7	11
Caloric density	++	+
	Cat <i>Felis catus</i>	Genet <i>Genetta genetta</i>
Total sleep	12.5	6.3
REM sleep	3.2	1.3
Brain/body ratio	0.009	0.012
Life span	13	13
Caloric density	+++	++
	Big brown bat <i>Eptesicus fuscus</i>	Greater short-nosed fruit bat <i>Cynopterus sphinx</i>
Total sleep	20	15
REM sleep	3.9	1.2
Brain/body ratio	0.0175	0.025
Life span	20	10
Caloric density	+++	+
	Burrowing owl <i>Athene cucularia</i>	Greylag goose <i>Anser anser</i>
Total sleep	14.3	6.4
REM sleep	0.7	0.7
Brain/body ratio	0.025	0.003
Life span	8	8
Caloric density	+++	+

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So why do most of us feel so poorly when we reduce our sleep time? Natural selection has imposed a certain amount of sleep on us to restrict activity to appropriate times of day and to reduce long-term nonvital energy expenditure. The pressure to sleep operates by reducing brain activity. Although individuals with naturally short sleep times are not at elevated risk compared to those with naturally long sleep times, repeated sleep deprivation below the body's programmed level is stressful and likely to impair health. Certain hormonal processes are linked to sleep. However, these are not universal, but rather are species and age specific (8, 12).

Sleep duration varies enormously across species, with total sleep amount ranging from 20 hours per day in the big brown bat to 2 hours per day in the horse. However, attempts to correlate sleep time with various parameters do not support any sleep physiology theory (8, 12). But species that eat food with low caloric density (e.g., herbivores) sleep less than those eating more nutritionally dense foods (e.g., carnivores) (12) (see the figure).

Quiet waking (with reduced motor activity) could serve the energy conservation functions attributed to sleep without the risks associated with the sleep state. However, the

brain consumes an inordinate percentage of metabolic energy during quiet waking, as much as 25% of the body's energy at rest. Brain energy consumption does not greatly differ between quiet and active waking, but it is greatly reduced in sleep (8). Animals will achieve a selective advantage in reducing brain energy consumption by sleep, but only if they have safe sleeping sites, such as underground burrows. Accordingly, large prey animals that do not have safe sleep sites do not sleep much and sleep very lightly (12).

In addition to mating and migration, sleep can also be reduced during food shortages, presumably to allow animals to invest more time in searching for the available food (13). Species whose environment has a severe seasonal variation in food availability have evolved to increase sleep during periods of food shortage and decrease sleep when food is available. Other species that have safe sites hibernate during periods of greatly reduced food availability, reducing energy expenditure even further (8).

The development of small devices that monitor sleep-related brain and muscle activity is likely to lead to major insights into the regulation of sleep duration under natural conditions (14). Darwin's evolutionary

monograph that underlies so much of our understanding of species survival does not consider the role of sleep in animal evolution. But understanding how the brain regulates sleep in response to behavioral requirements and environmental conditions across species will be a fruitful new paradigm for both sleep research and evolutionary biology.

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ASTRONOMY

How Oblate Is the Sun?

Douglas Gough

At the end of the 19th century there was much concern over Le Verrier's realization that the orbit of Mercury differed from that expected from Newtonian physics. After taking account of perturbations from other planets, there was an unexplained residual precession of the elliptical orbit that amounted to just 43 arc sec per century. It was pointed out by Newcomb that this residual precession might be explained by the Sun being oblate. Einstein then demonstrated that his new general theory of relativity accounted for almost all of the precession, assuming the Sun to be precisely spherical. Only a small 0.2% of the original discrepancy then remained to be explained otherwise, presumably by an oblateness caused by rotation of the Sun. The most

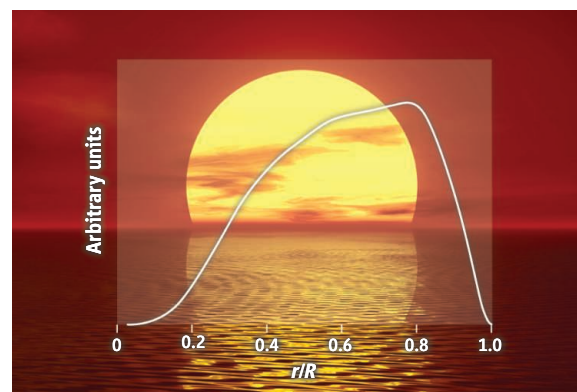
natural way to determine the oblateness is simply to measure the apparent shape. However, despite many attempts over more than a century, that has not been possible with the required precision. The reason is that

Recent measurements show that the Sun appears to be rounder than current understanding predicts.

ground-based observers must contend with variations in the refractive index of Earth's atmosphere, which distort the image of the Sun. Only with instruments in space has it been possible to approach a useful measurement (1).

On page 1638 of this issue, Kuhn *et al.* (2) present results from the Heliospheric and Magnetic Imager (HMI) on NASA's Solar Dynamics Observatory (3), indicating that the Sun appears not to be as flattened as it should be.

The visual oblateness of the Sun, defined as $\Delta_v = (R_e - R_p)/R$, where R_e and R_p are the equatorial and polar radii and R the mean radius, can be separated into two parts. One is the direct distortion $\Delta\Omega$ due to the pull on the surface layers by the centrifugal force, which



Solar distortion. Superposed on the setting Sun is a graph depicting by how much Ω^2 contributes to the distortion of the Sun's gravitational field as a function of distance from the center (14).

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is proportional to the square of the angular velocity Ω and which acts outward from the axis of rotation to make the equator bulge; the other is the aspherical deviation $\Delta\Phi$ of the gravitational potential produced by the centrifugal force distorting the inside of the Sun, principally at mid-radii (see the figure). From the angular velocity of the visible surface (4), the former is determined to be $\Delta_\Omega \sim 8.4 (\pm 0.2) \times 10^{-6}$. It was then expected that $\Delta\Phi$ could be obtained by simply subtracting Δ_Ω from Δ_v . Had the entire anomalous precession been due to a solar oblateness, $\Delta\Phi$ would have dominated the full oblateness, and the procedure would have been viable. But general relativity indicated that $\Delta\Phi$ is much smaller, and helioseismic observations (5) indicate that it is only 3.3×10^{-7} , just 4% of Δ_Ω , thereby implying that Δ_v should be 8.7×10^{-6} .

The first of the modern observations were made serendipitously (1) using the guiding sensor for the Reuven Ramaty High-Energy Solar Spectroscopic Imager (RHESSI) (6). A rapidly rotating optical image of the solar limb was Fourier-analyzed with respect to the angle about the line of sight to estimate the quadrupolar component of the limb displacement. A major problem with the measurement, as with any other limb-shape measurement, is brightness contamination by sunspots and magnetically generated excess emission. In an attempt to eliminate the detritus, a magnetic-activity proxy was adopted to reject suspect data (7). The outcome was $\Delta_v = 8.34 (\pm 0.15) \times 10^{-6}$.

Kuhn *et al.* present an analysis of limb-brightness data in a photospheric Fe spectral line obtained from HMI. The limb-darkening function was measured as a function of the angular orientation of the Sun's image at times when the spacecraft was rotated for calibration purposes about its pointing axis. Magnetic contamination was removed by rejecting limb-position data associated with brightness above some threshold that had been judged to yield a robust outcome. Detailed analysis (8) then yielded $\Delta_v = 7.56 (\pm 0.40) \times 10^{-6}$.

Note that the uncertainties in the values of Δ_v are not significantly smaller than $\Delta\Phi$. Therefore, subtracting Δ_Ω from Δ_v is evidently not a viable procedure for determining $\Delta\Phi$ (9, 10). More startling, however, is that Kuhn *et al.* found Δ_v to be some 10% lower than the value of Δ_Ω expected from the surface rotation. Therefore, if their observations are correct, something else other than rotation must be affecting the limb. Until that is explained and quantified precisely, solar limb shape cannot provide a means

to test gravity; helioseismology is, and will remain, the only accurate tool to do so.

The new oblateness measurements beg explanation. An expected polar temperature excess comes to mind (11); however, Kuhn's (12) estimate of the excess is just 1 to 2 K, which is grossly insufficient to explain the discrepancy. Alternatively, one could wonder whether polar support is boosted by an intense large-scale surface magnetic field; might not the intensity of the field demanded for that be plausible if the spatial variation in the differential rotation requires forces, perhaps magnetic, of sufficient strength to produce a shape distortion of similar magnitude to that produced directly by the differential rotation itself? That is not necessarily so, as any child in a playground who has spun up a merry-go-round with ease but then had difficulty holding on against the resulting centrifugal force well knows. Even if a magnetic hill were present, the surface material would surely drain away. What other possibility is left? Turbulent stresses from convection, perhaps? These newest data have left us with an intriguing new conundrum in solar physics: Why does the Sun appear to be so round?

ECOLOGY

Insecticide Resistance After *Silent Spring*

David G. Heckel

Combating insecticide resistance is a continual challenge for the preservation of both traditional and transgenic crops.

Rachel Carson's book *Silent Spring*, published 50 years ago (1), eloquently awakened the public to the manifold dangers for the environment and human health posed by the wanton use of chemical pesticides (2). Carson argued that in addition to their many harmful ecological effects, chemical insecticides ultimately undermine sustainable pest management: They kill the parasites and predators that formerly held many pests in check, while the pests themselves become resistant and require ever-higher amounts of sprays for their control. Since the publication of *Silent Spring*, more than 450 arthropod

species have been reported with resistance to one or more pesticides (3). Yet over the same period, a paradigm shift in dealing with this global problem has also occurred.

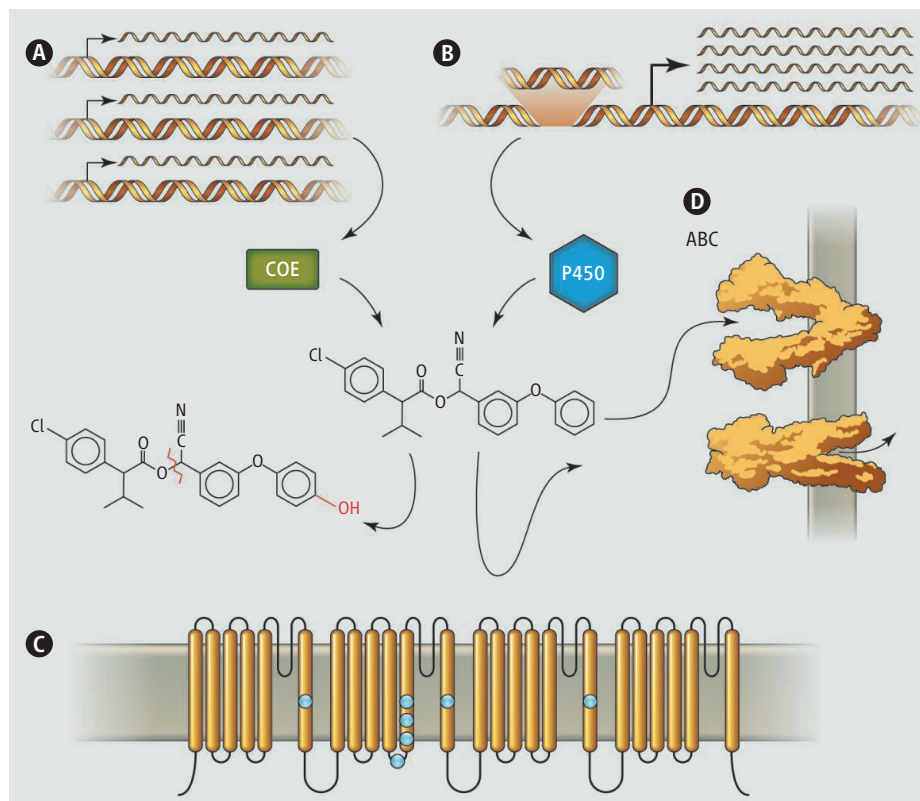
Resistance, defined as the heritable decrease in a population's susceptibility to a toxin to which it is exposed over successive generations, is an example of evolution by natural selection. The intensity of selection can be controlled by varying the insecticide and reducing the frequency and intensity of application. A variety of different chemical classes has been developed, targeting a range of biological targets in the insect. Yet because the number of targets is still limited and new targets are not resistance-proof, prolonging the useful life of existing insecticides by judicious use has increasing priority in their

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Some mechanisms of insecticide resistance. Insects have evolved mechanisms to detoxify, reduce their sensitivity to, or excrete insecticides. Increased detoxification can occur by (A) gene duplication of carboxylesterase (COE), which cleaves the insecticide, or (B) transposon insertion, causing increased transcription of P450, which hydroxylates the insecticide. (C) Point mutations in the target can reduce insecticide binding. (D) Increased transporter activity leads to faster excretion from the cell.

commercialization (4). Because pesticide use is difficult to control on a global level, resistance management programs must be locally suitable, economically feasible, and voluntarily adopted by growers.

Pesticide resistance often results from gene regulatory changes, which lead to an increase in the efficiency of one or more physiological systems used by the insect for detoxification: oxidation, conjugation to hydrophilic compounds, and excretion (see the figure). Constitutive up-regulation of these detoxifying enzymes is most common, although gene amplification is another mechanism for increasing the amount of protein available to inactivate the insecticide. In such cases, general inhibitors of classes of detoxifying enzymes may be used to counter the increased detoxification ability.

Rarely, a single mutation can confer a novel detoxifying ability, such as the substitution of an aspartate for a glycine in a carboxylesterase of the Australian sheep blowfly, converting it to an organophosphorus hydrolase (5). More commonly, single mutations reduce the sensitivity of the insecticide's biochemical targets, including enzymes and ion channels in the nervous system. In these pro-

teins, which are essential for life, only a very few mutations are compatible with biological function; these mutations are often found in many different species exposed to the same insecticide, representing multiple cases of parallel evolution. Thus, engineering modified insecticides that are more potent on the altered target sites could be of general benefit. This predictability at the level of target-site mutations is, however, often frustrated by the presence of additional, more diverse detoxifying mechanisms in the same species.

A good example is the complexity of resistance in mosquitoes, which are vectors of malaria and other diseases. Gene amplification causing overproduction of organophosphorus-inactivating carboxylesterases has spread worldwide in *Culex* species (6). Some *Culex* species have evolved further to replace this energetically costly overproduction of protein by mutation and gene duplication of the target, acetylcholinesterase (AChE) (7). Bariami *et al.* recently used microarrays to show that increased expression of a CYP9J-type P450 and an ABC transporter, partly due to gene amplification, may underlie resistance to pyrethroids in populations of the dengue vector *Aedes aegypti* (8).

The aphid *Myzus persicae* is another serial offender. It first evolved organophosphorus resistance by making up to 80 copies in its genome of a pair of esterase genes, resulting in esterase protein up to 1% of body weight (9). This expensive overproduction is switched off by an unknown mechanism when the aphid demethylates these gene copies. Target-site mutations in AChE compensate for this loss; sodium channel mutations additionally confer pyrethroid resistance. The aphid counters the newer neonicotinoid insecticides by multiple duplications of the CYP6CY3 P450 gene (10) and a target-site mutation in a subunit of the acetylcholine receptor (11); this combination of mechanisms is especially potent.

The spider mite *Tetranychus urticae* is notorious for rapidly evolving resistance, spurring the development of novel chemicals for control. Resistance to binfenazate has been shown to result from four mutations in the mitochondrial DNA that encodes cytochrome b; these mutations occurred at positions that are otherwise completely conserved across eukaryotes, identifying the target site of this new pesticide (12). This unusual, maternally inherited resistance responds extremely rapidly to selection in the field. Recently, Van Leeuwen *et al.* used the mite's genome sequence to investigate resistance to etoxazole, which inhibits synthesis of the chitinous exoskeleton of arthropods. By selecting a mixed population and tracking frequency changes of more than 700,000 polymorphisms, the authors identified a genome region that contains a mutated chitin synthase gene, with a single amino acid substitution conferring resistance (13).

Toxicity to nontarget organisms and reduced effectiveness on resistant pests constituted two separate threads of the argument against pesticides in *Silent Spring* (1). Remarkably, these ideas intersect on the genetic model *Drosophila melanogaster*, which, despite not being a pest, has received enough incidental environmental exposure over the years to develop resistance to several older insecticides. Dieldrin-resistant flies from the wild enabled the first identification of the *Rdl* mutation in the GABA-gated chloride channel (14), subsequently found in many pest species. One cause of DDT resistance is the insertion of a transposable element in the promoter of CYP6G1 (15), leading to overexpression of this P450 enzyme, which detoxifies DDT. *Drosophila* later attained even higher DDT resistance by two additional insertions and a gene duplication, revealing an ongoing process of multiple adaptive steps (16). To identify

the targets of newer insecticides, Perry *et al.* have used mutagenesis of *Drosophila* to create strains resistant to spinosad or neonicotinoids; they pinpointed the acetylcholine receptor subunits that are most sensitive to these toxins (17).

The goal of reducing the use of chemical insecticides has spurred the search for biologically based alternatives, a strategy encouraged by Carson [chapter 17 in (1)]. Insecticidal protein toxins from the bacterium *Bacillus thuringiensis* (Bt) are now expressed in more than 58 million hectares of transgenic cotton and maize worldwide to deter lepidopteran pests (18). When Bt cotton was first introduced in the United States and Australia, government-mandated, industry-implemented resistance management plans were in place. These “high dose/refuge” strategies aimed to slow the process of natural selection, first by ensuring that transgenic plants expressed enough toxin to kill all but the most resistant insects, and second by providing non-Bt crops as “susceptibility refuges” on which Bt-susceptible pests could develop to adulthood and mate with the relatively few survivors from the Bt crop. These strategies to delay resistance are working so far in most cases (19).

What if they fail? Estimation of the frequency of rare Bt resistance alleles before they become common enough to cause unsustainable crop damage can provide

advance warning of developing resistance. Using methods based on the inbreeding of large field samples, Downes and Mahon have detected alleles for resistance to the Cry2Ab toxin at frequencies of 0.5 to 0.9% in two species of bollworms in Australia (20). When the resistance gene is known, DNA sequencing can also be used; Zhang *et al.* have correlated mutations in a 12-cadherin-domain protein with bollworm resistance to the Cry1Ac toxin in China (21). Modified Bt toxins have been engineered to circumvent this type of resistance and show promise on other Bt resistance mechanisms as well (22).

Coexpression of an additional toxin, Vip3A, with a different mode of action has been commercialized to delay pest resistance to transgenic crops; however, the Vip3A-resistant allele frequency is already 2.7% in one pest, which is very high given that there has been no prior exposure to this toxin (23).

Forewarned by the long history of insecticide resistance, the deployment of transgenic crops for insect control has incorporated resistance management plans from the beginning. Unfortunately, this has not been the case for transgenic crops engineered for herbicide tolerance. Greatly increased spraying to control weeds in these new crops has led to a rapid rise of herbicide resistance in several weed species (24), and agronomists must now follow entomologists in learning the hard lessons of the past 50 years.

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ECOLOGY

Life in a Contaminated World

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Until the early 1960s, pesticide use was perceived as a benefit to agriculture and public health, with few detrimental consequences. This perception changed dramatically with the publication 50 years ago of Rachel Carson's *Silent Spring* (1). The book was the start of a debate that continues to this day on the relative benefits and risks of not just pesticides but all synthetic chemicals.

Pesticides are unquestionably beneficial for food production (2), but there is a growing awareness of the risks to human and ecological health associated with their use. Over the past decade, a growing literature (3–6)

has examined how early-life exposure to an array of chemical agents, found not only in pesticides but also in personal care products and plastics, can affect human health. The effects on endocrine signaling (and thus endocrine disruption) have been observed in the exposed generation and also in succeeding generations, but the conclusions are not without controversy.

“It is ironic to think that man might determine his own future by something so seemingly trivial as the choice of an insect spray” wrote Carson in 1962 [p. 8 in (1)]. Although she had no mechanism to explain her observations, it is now well documented that exposure early in embryonic development to commonly used chemicals alters gene expression patterns that can lead to altered health later in life (7).

Exposure to pesticides and other chemicals can have complex long-term health effects.

But what dose is required to cause an effect? A large literature in the fields of endocrinology and general physiology demonstrates not only that different effects can be induced at different doses but also that the mechanisms driving those effects can differ as well (7). A report from the Endocrine Society states that different effects should be expected when comparing high- and low-dose regimens of endocrine disruptors (3). Studies using acute high-dose exposures may thus be of limited value for predicting what might occur following the chronic low-dose exposures that almost every population on Earth is subjected to today, often at low but detectable concentrations.

Early-life exposure to chemicals with endocrine disruption potential has been shown to alter gene expression profiles that

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are linked to altered morphology and physiology, such as compromised fertility and reproductive tract development, altered metabolism, obesity, and altered behavior (8–11). The multigenerational relationship between chemical exposure and health has been observed in laboratory models and wildlife (4). Given the heritable nature of some epigenetic modifications (termed germ line–dependent epigenetic modification) (12), the results indicate that the classic “gene by environment” paradigm used to understand environmental impacts on health is incomplete: The parental genome is based on both the genome inherited from the parents (which includes accumulated gene mutations) and the epigenetic modifications that occurred to that genome before fertilization of the new offspring (see the figure).

Much has been made of the inability of some research groups to replicate the endocrine disruptive effects of some chemicals reported by other laboratories (13–15). For example, Hayes *et al.* (16) reported effects on the development of frogs after exposure to environmentally relevant concentrations

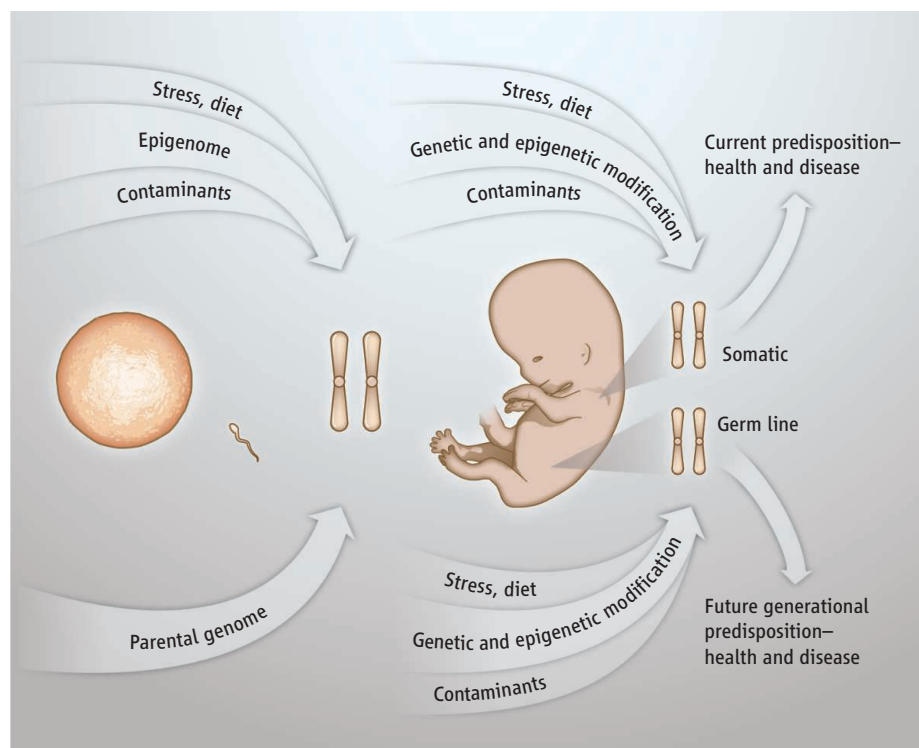
of atrazine, but other groups were unable to replicate these findings (15, 17). However, differences in the design of these experiments did exist, including the source of the animals used and the density at which they were housed (18). Disparate outcomes have also been reported in studies of bisphenol A in rodents that used different designs or methodologies (19) and in studies of human semen quality or genital development (20).

Blount *et al.* have found that in men, perchlorate (a contaminant that affects thyroid function) showed no relationship with the concentrations of thyroid biomarkers. In contrast, in women, raised urinary iodine levels were associated with an influence of perchlorate on thyroid biomarker concentrations (21). Thus, dietary iodine, a key factor influencing urinary iodine output, influenced whether an effect was observed or not. How many studies examining potential effects of contaminants on thyroid function record the iodine concentration in the diet of their research animals?

Other studies also illustrate the complexity of the response to environmental endo-

crine disruptors. For example, Spearow and colleagues have shown in a series of studies that differing strains of mice respond dramatically differently when exposed to the same estrogenic drugs or doses (22). Further, a physical factor such as hypoxia can down-regulate the mixed-function oxygenase enzyme (CYP1A) that metabolizes polycyclic aromatic hydrocarbons and polychlorinated biphenyls, thereby affecting the biotransformation of environmental contaminants, thus altering persistence or even the metabolites present (23). These complex genetic and environmental effects must be taken into account in studies assessing the health effects of environmental pollutants.

Rachel Carson was right: Chemical contaminants play an important role in our health and the health of the environment. We must continue her legacy and focus on how exposure to environmental contaminants, stress, and diet interacts with the human germline genome and epigenome to establish predispositions for disease that are influenced by secondary exposures later in life (5). Understanding this complexity is essential to our understanding of the multiple roles of the environment in promoting those factors that lead to health.



The role of the environment. Environmental factors, including numerous contaminants, have been shown to modify the parental genome, so that the genetic makeup of any offspring is a combination of a parental inherited genome (itself likely influenced by epigenetic mechanisms of the germ line) and environmental influences on that germ line during maturation. Environmental factors such as diet, stress, and contaminants can also modify the genome of the developing embryo by classic selection and mutation or by epigenetic mechanisms at both the somatic and germline levels. These modifications can produce predispositions for health and disease in the current lifetime of the individual. Future transgenerational effects could also be established through modifications in the germline genome or epigenome after exposures during the lifetime of that individual.

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CANCER

Immune Surveillance from Chromosomal Chaos?

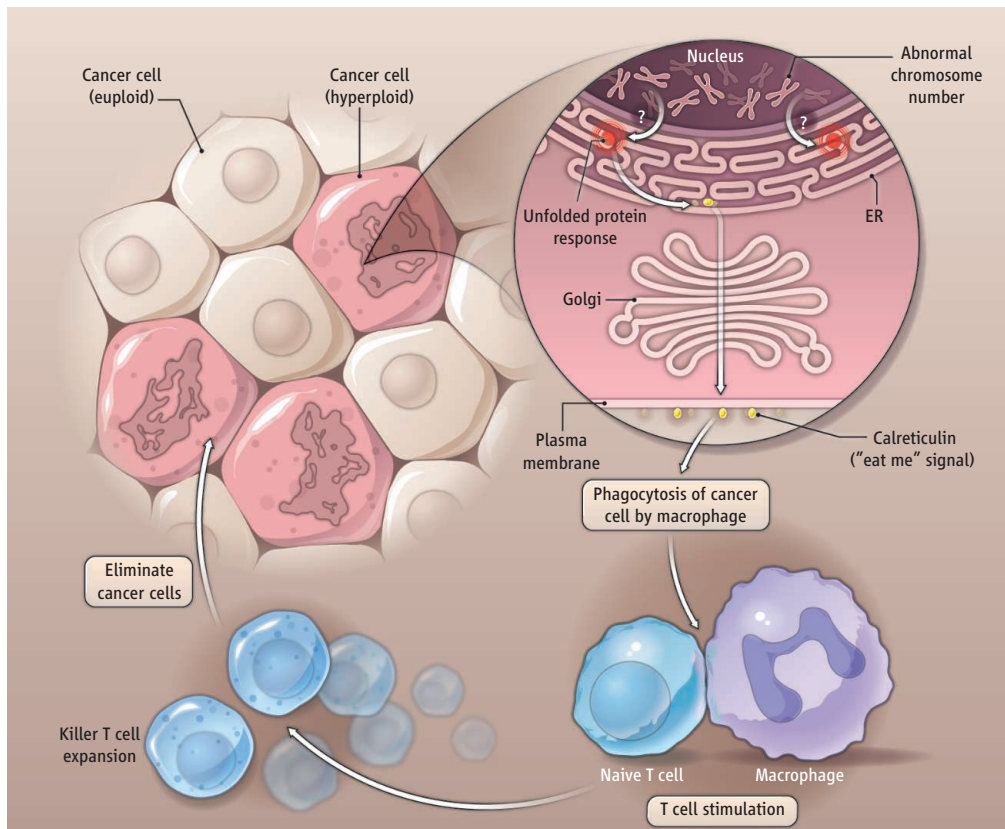
Maurizio Zanetti and Navin R. Mahadevan

In 1970, the cancer immune surveillance hypothesis proposed that “when aberrant cells with proliferative potential arise in the body, they will carry new antigenic determinants on their surface. When a significant amount of new antigen has developed, a thymus-dependent immunological response will be initiated and eventually eliminates the aberrant cells” (1). This idea stirred much debate, and it remains unclear whether T cells spontaneously reject tumors or select variants of low immunogenicity. Still, the hypothesis is being revisited and revised with new insights into the dynamics of cancer cell immunogenicity in the context of tolerance and other elements of the antitumor immune response (2). Adding to this, Senovilla *et al.* (3) report, on page 1678 of this issue, that there is a connection between abnormal chromosome number and the immune surveillance of such aberrant cancer cells.

Cancer cells and their progenitors have long been known to have abnormalities in the number and visual appearance of their chromosomes. In the early 1900s, it was suggested that normal cells become malignant when they acquire chromosomal abnormalities (e.g., aneuploidy and hyperploidy) (4). This idea had little traction for most of the 20th century, but over the past decade, the debate has reopened (5). The most recent prevailing view is that carcinogenesis arises from genetic instability such as loss of a suppressor gene and activation of an oncogene (6). In support of the former view, carcinogenesis has been shown to be initiated by random aneuploidy, which is itself generated by a carcinogen or spontaneously and precedes genetic abnormalities (7).

This renewed debate raises an intriguing question about whether there is a connection

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Order from chaos. Hyperploidy in cancer cells activates the unfolded protein response in the endoplasmic reticulum (ER), which promotes the export of the ER-resident protein calreticulin to the cell surface where it elicits phagocytosis by macrophages and dendritic cells. These, in turn, present cancer cell antigens to T cells, driving their clonal expansion. The resulting killer T cells preferentially attack hyperploid cells, leading to attenuation or arrest of tumor growth.

between abnormal ploidy and immune surveillance. Senovilla *et al.* report that hyperploid cancer cells become immunogenic as the result of a constitutive endoplasmic reticulum (ER) stress response, which results in the aberrant cell surface display of calreticulin. Calreticulin is an ER-resident protein that prevents the egress of misfolded proteins from the organelle. Previous work showed that calreticulin presented at the cell surface dictates the immunogenicity of cancer cells undergoing programmed cell death (apoptosis) (8), thus serving as an “eat me” signal for phagocytic cells, macrophages, and dendritic cells. More recently, an inhibitory “do not eat me” cell surface signal, CD47, was shown to hinder the antitumor immune response (9). Senovilla *et al.* now show that chemical inhibitors of cytokine-

sis and microtubule formation induce polyploidization, which promotes the export of calreticulin to the cell surface through activation of the ER stress response. This display of calreticulin triggers immune rejection of polyploidy cells (see the figure).

In mammalian cells, the ER stress response, also known as the unfolded protein response (UPR), is a checkpoint in the biosynthesis, folding, assembly, and modification of membrane-bound and secreted proteins (10). It is initiated by three ER membrane-bound sensors: IRE1 α , ATF6, and PERK. The accumulation of unfolded or misfolded proteins in the ER causes these sensors to bind to aberrant proteins and activate signaling pathways to normalize protein folding and secretion (11). This includes phosphorylation of the eukaryotic initiation factor eIF2 α by PERK,

which blocks the translation of messenger RNA to protein and thereby reduces the protein load in the ER. If ER stress persists, signaling from PERK can also stimulate the transcription factor C/EBP Homologous Protein (CHOP), which can promote inflammatory signaling or initiate apoptosis. Thus, if ER stress is too intense or protracted, compensatory mechanisms fail and cells die (11).

Remarkably, the injection of hyperploid cancer cells into syngeneic immunocompetent mice yielded tumors that appeared less frequently, or grew more slowly, than when immune-incompetent mice were inoculated—an effect that depended on calreticulin. Furthermore, the immune response elicited by injection of calreticulin-positive cancer cells exerted selective pressure on the tumor by eliminating hyperploid cells. Senovilla *et al.* suggest that cancer cells may be kept in check by virtue of their chromosomal content. In their retrospective analysis of 60 breast cancer patients who had received chemotherapy, the authors observed that cancer cells from patients who responded to chemotherapy (responders) had reduced nuclear size, which they interpret as less chromatin mass and an indirect indication of less hyperploidy. Biopsies showed an increased ratio of killer (CD8⁺) T cells (which target cancer cells specifically and eliminate them) to suppressor/regulatory T cells (which counteract killer T cell responses, hence hindering immune-mediated protection). These features were absent in nonresponder patients. However, differences in ploidy between responders and nonresponders were not interrogated.

A link between genome integrity and the immune response was previously shown in which the activation of a DNA damage response by genotoxic stress induced the overexpression of cell surface markers that render cancer cells more susceptible to attack by natural killer cells of the immune system (12). Senovilla *et al.* suggest that T cell immunity can be regulated by a hyperploidy-mediated UPR. A major question is the nature of the signal that activates the UPR in hyperploid cancer cells. One possibility is that hyperploidy may increase protein synthesis as a result of the higher number of genes in the cell, which could cause ER stress. The role of PERK signaling in modulating immune surveillance is also an intriguing question. Oddly, eIF2 α phosphorylation in biopsies from nonresponder breast cancer patients was unchanged or increased relative to responders, and biopsies from nonresponders showed increased eIF2 α phosphorylation after treatment. This indicates

that although cancer cells resistant to chemotherapy have an active PERK-mediated UPR, they unexpectedly fail to engage the immune system. Perhaps molecules that interfere with the PERK-eIF2 α axis could be considered as treatment for chemoresistant tumors. The finding of Senovilla *et al.* raises the provocative idea that hyperploidy changes the repertoire of antigens expressed and presented by cancer cells, rendering them selective targets of killer T cells.

Another consideration is the UPR, as it affects the expression of genes associated with immunity, such as the activation of pro-inflammatory responses, as well as tumor-promoting cytokine genes (13). In tumor cells, the activated UPR is the origin of cell-extrinsic effects that imprint myeloid cells (macrophages and dendritic cells) with a pro-inflammatory, suppressive phenotype (14). Such overt inflammation at the time of T cell priming could severely hamper the formation of T cell memory (15); thus, adaptive antitumor T cell immunity may not be long-lasting, thereby compromising immune surveillance.

The intriguing idea that the UPR increases immunogenicity and mediates the ostensible elimination of hyperploid cancer cells expands our knowledge about the

complex relationship between cancer cells and the immune system. It must, however, be integrated into the larger context of the tumor-immune system interface. As Plato elaborated in *Timaeus*, the universe formed through order emerging from chaos. It seems that the connection between immune surveillance and chromosomal chaos follows this very principle.

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BIOCHEMISTRY

A Radical Route for Nitrogenase Carbide Insertion

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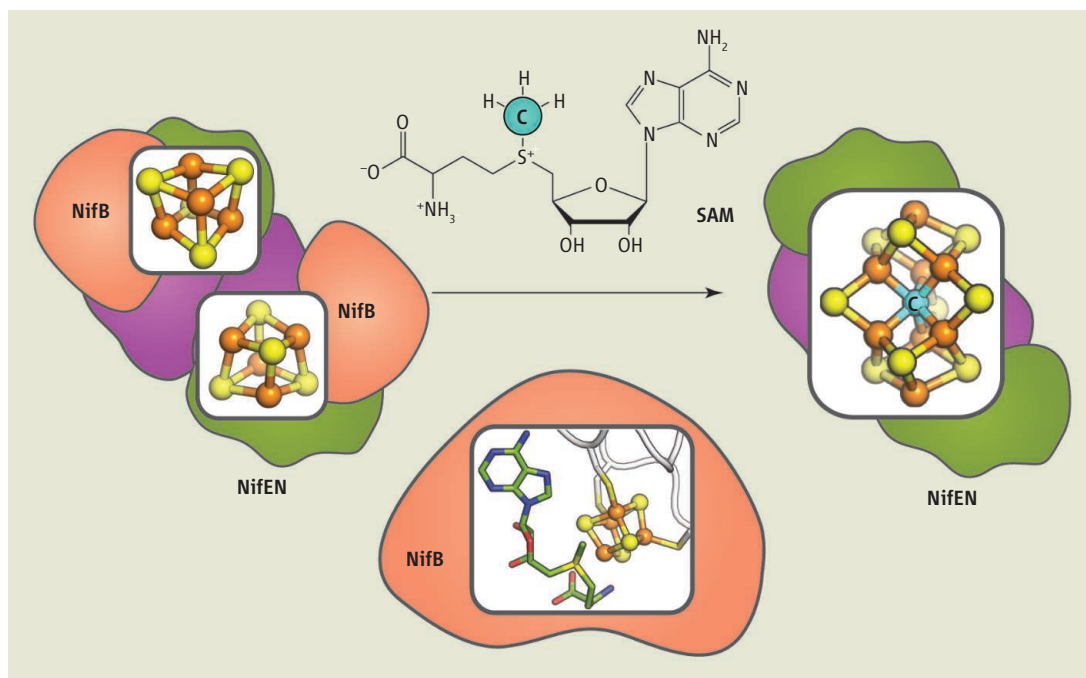
The NifB protein inserts a carbon atom from S-adenosyl-L-methionine into the complex iron-sulfur cluster cofactor of the nitrogenase enzyme.

Nitrogenase is the only known biological catalyst for the conversion of dinitrogen (N₂) to ammonia (NH₃), a reaction that provides the “fixed” nitrogen required by most organisms. Biological breakdown of N₂ by bacterial nitrogenase enzymes occurs at a complex metallocofactor consisting of seven iron (Fe) ions, nine sulfides (S²⁻), a molybdenum (Mo) ion (most commonly), and a homocitrate ligand (1, 2). Twenty years ago, crystal structures of nitrogenase provided the first glimpses of this FeMo cofactor, also called the M cluster, revealing that it resembles two [4Fe-4S]

cubes (a more ordinary type of iron-sulfur metallocluster) linked by an additional sulfur atom and with one Fe ion replaced by the Mo ion (3). Ten years ago, near-atomic resolution crystallographic data revealed an atom in the middle of the cluster, bonded to six of the Fe ions (4). Its identity remained under debate until late last year, when two separate groups identified it as a carbide ion (5, 6). Remarkably, less than a year later, Wiig *et al.* (7) on page 1672 of this issue identify both the enzyme that inserts the carbon, NifB, and the molecule that supplies it, the ubiquitous biological carbon donor S-adenosyl-L-methionine (SAM).

The observation of a central C atom in the M cluster places it among a select few biological metal centers that contain an Fe-C

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Enter the carbon atom. A radical SAM enzyme inserts carbon into the nitrogenase catalytic cofactor. Wiig *et al.* show that the interstitial carbon atom in the M cluster is inserted by NifB and derives from SAM. NifB joins a larger group of radical SAM enzymes that use SAM both to perform radical reactions and as a methyl donor but may use a different chemical mechanism. Iron and sulfur atoms are shown as orange and yellow spheres, and the interstitial carbon is shown in cyan. SAM in NifB is shown in stick representation.

form Fe-C bonds, is fundamentally different from the known radical SAM methyltransferases (9, 11).

The discovery by Wiig *et al.* that SAM donates a methyl group

during nitrogenase assembly and that the associated C atom ends up in the active M cluster validates the identity of the once-enigmatic interstitial atom and solves the mystery of its origin. There are many outstanding questions about the exact mechanism of C-atom incorporation as well as about M-cluster assembly in general. How is the methyl group moved from SAM to the assembly machinery? What is the source of the extra S atom? What are the details of Fe-C bond formation and deprotonation to carbide? In addition, the role of the carbide in N_2 fixation remains unclear and adds a new dimension to ongoing studies of the nitrogenase mechanism. Given the complexity of the nitrogenase catalytic cofactor, the details of how it is put together and how it activates N_2 will likely involve exciting and novel chemistry.

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bond and adds a new layer of complexity to its biosynthesis. Nitrogen-fixing bacteria use an array of proteins to assemble and maintain a functional nitrogenase enzyme (8). Three proteins, NifH, NifE, and NifB, play a direct role in M-cluster synthesis. NifH performs the last step, the insertion of Mo into an M-cluster precursor that initially resides on NifE, which serves as a scaffold for cluster assembly. NifE delivers the mature M cluster to the catalytic component of nitrogenase, called NifDK. NifB assembles the Fe-S core of the cofactor, which is proposed to involve stitching together two [4Fe-4S] clusters with an additional S atom. NifB belongs to the radical SAM enzyme superfamily, proteins characterized by a Cys- X_3 -Cys- X_2 -Cys signature motif (where X denotes any amino acid) that use SAM to mediate diverse anaerobic chemical transformations involving radicals (atoms or molecules with unpaired electrons) (9). NifB initially eluded biochemical characterization because, like nitrogenase itself, it contains Fe-S clusters that degrade upon exposure to oxygen. However, a clever fusion of NifB to NifE recently allowed spectroscopic detection of [4Fe-4S] precursor clusters that can be coupled into an 8Fe cluster in the presence of SAM (10).

Using this combined NifE-NifB protein, Wiig *et al.* now show that NifB and its SAM cofactor are involved in M-cluster carbon insertion as well as Fe-S cluster assembly. The first clue came from analysis of the SAM-derived products of the reaction between NifB and SAM. Two different compounds were observed, one consistent with

the generation of a 5'-deoxyadenosine radical (5'-dA•) and the other with carbon donation. Experiments with labeled versions of SAM provided more details about the reaction. The C atom ultimately mobilized to the M cluster starts off as the SAM methyl group (-CH₃), and the 5'-dA• removes an H atom from the methyl group, presumably generating a transient -CH₂• radical as part of the conversion to an Fe₆-carbide species. Most important, Wiig *et al.* used a radioactive assay to specifically track the C atom from its origin in the SAM methyl group all the way through the M-cluster biosynthetic pathway, unequivocally showing that the C atom remains associated with the cofactor after insertion into nitrogenase.

Catalysis by NifB of two different SAM-dependent reactions reveals a new connection to a larger group of radical SAM enzymes that also use SAM as both a methyl group source and an initiator of radical reactions (11). The most comprehensively studied enzymes in this class, a pair of ribosomal RNA (rRNA) methyltransferases named RlmN and Cfr, have unusual mechanistic features that include using a conserved cysteine residue in the protein as a methyl carrier and a single SAM binding site for both the methyl transfer and radical reactions (12, 13). Whether NifB exploits any of these strategies remains to be seen, but methyl modifications indicative of a carrier residue have not been detected, suggesting that it may work differently. A distinct mechanism would not be surprising because NifB, which uses a methyl group to ultimately

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RETROSPECTIVE

R. Duncan Luce (1925–2012)

James L. McClelland

Robert Duncan Luce, a mathematician who sought to provide axiomatic formulations for the social sciences, died on 11 August 2012 in Irvine, California, at the age of 87. His passing was marked by an outpouring of sadness at the loss of a revered colleague and expressions of veneration for his many substantive, institutional, and personal contributions to the mathematical social sciences.

Luce was a mathematician before he was a social scientist. His achievements cut across the political, social, economic, and social sciences for over 60 years. These contributions were recognized early in his career, with election to the U.S. National Academy of Sciences in 1972, and later at the highest level, with the U.S. National Medal of Science in 2003.

Luce received his Ph.D. in mathematics from the Massachusetts Institute of Technology (MIT) in 1950, with a thesis that addressed a topic in abstract algebra. His first publication applied ideas from this field to provide a mathematical definition of a “clique” within a social network, and explored using a matrix to capture the connections among individuals that might also be represented in a graph. This method has become standard in computer science, with applications across the social sciences and many other branches of science.

In the 1950s, Luce tackled the problem of choice. His seminal treatment of this issue in his 1959 book, *Individual Choice Behavior: A Theoretical Analysis*, provided the foundation for a vast range of investigations in psychology and economics. In essence, he proposed an abstract and general axiom that gives rise to the following principle of choice: The relative probability (p) of choosing item a or item b is independent of other available choices. Suppose in a choice between an apple and a banana, you choose the apple two-thirds of the time, and the banana one-third of the time, for a probability ratio of 2:1. If I add a third choice (a pear), then you might sometimes choose the pear, but the ratio of apple to banana choices should still be 2:1. Luce showed that if his general axiom is true, we can define for each item a posi-



tive strength S , such that whatever the set of alternatives, $p(a)/p(b) = S(a)/S(b)$. Luce's theory was important, not because it was true—although there are circumstances in which it is approximately true—but because its success or failure can lead to insight into the processes underlying choices and decisions. A great deal of work since Luce's theoretical analysis explores the situations in which his theory may be right or wrong.

In the 1960s, and for the rest of his career, Luce's focus shifted to fundamental questions of measurement, with a particular emphasis on measuring psychological quantities such as value or loudness. Social scientists distinguish between physical quantities such as units of sound intensity or ounces of gold, on the one hand, and psychological variables such as perceived loudness and value or utility on the other; the behavioral and economic actions of individuals, groups, and organizations depend on the psychological quantities involved. From the 1960s onward, Luce sought to establish fundamental principles relevant to the measurement of these psychological quantities. In joint work with the statistician John Tukey in 1964, Luce showed that experiments simultaneously varying the effects of two factors on a simple response measure can allow the establishment of unique functions characterizing the effect of each factor—provided, as in the choice work, some simple and intuitively plausible axioms apply. For example, suppose we create batches of cookies, varying the size of each cookie in the batch and the number of cookies in the batch; we

A pioneer in the social sciences combined mathematics and psychology to understand human behavior.

present people with choices between two batches, each with a different combination of cookie size and cookie number; and we simply ask each participant to say which batch they would prefer. Then, if the axioms hold for their responses (and if we collect sufficient judgments), we can reconstruct unique functions indicating how size and number affect perceived desirability of a batch of cookies. In this way, psychological forms (and other forms) of measurement can proceed with nothing more than comparative judgments of desirability. This is useful because explicit estimates of perceived value are easily influenced and may not measure the factors that determine preference when faced with a real choice between alternatives. The approach is widely used in perceptual psychology, behavioral economics, and throughout the social and decision sciences. Luce continued to reflect on the fundamental measurement issues until the end of his career, collaborating with other mathematically inclined social scientists, including Louis Narens and Patrick Suppes.

Luce's career took him to many institutions, including Harvard University, Columbia University, the Institute for Advanced Study at Princeton, the University of Pennsylvania, and the University of California, Irvine. In 1988, he began a second stint at Irvine as a Distinguished Professor of Cognitive Sciences and Economics to found the Institute for Mathematical Behavioral Sciences, where he explored the foundations of measurement.

A generous supporter of colleagues and a careful mentor, Luce contributed in many quiet ways to advance the goals of the social and behavioral sciences. He was instrumental in creating the *Journal of Mathematical Psychology*, and played a lead role in several multivolume works, including the *Handbook of Mathematical Psychology* and the *Foundations of Measurement*.

Luce saw the marriage of mathematics and behavioral science as a gamble. After receiving the National Medal of Science from President George W. Bush, he said, “I guess that 1950 gamble back at MIT paid off.” It certainly paid off for our understanding of human behavior.

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IBI* SERIES WINNER

Personal Plants: Making Botany Meaningful by Experimentation

Laura A. Hyatt

The use of scientific inquiry in studying plant growth is perhaps as old as agriculture itself. Our ability to feed our current population is the result of the historical application of the scientific method to basic questions about the response of plants to their environment. However, most of today's students have likely never pondered basic questions about plant growth and may not even know general rules of thumb about growing their own food.

The importance of understanding plant life is increasing. Plants play critical roles in maintaining the carbon balance in the atmosphere, and they form the base of food pyramids throughout the world (1, 2). Food, water, and energy shortages can also be linked to plants (or a lack thereof). Low botanical diversity in diets is a major contributor to the decreasing quality of human nutrition, linked to the proliferation of a multitude of health problems (3). It is essential that students learn to perceive plants as partners in the continued functioning of a healthy planet and society.

The Personal Plant Project at Rider University addresses these needs using an inquiry-based approach that guides students to an understanding of the rationale behind a variety of basic principles of plant culture while simultaneously teaching them about the scientific method through application. This semester-long curriculum provides multiple avenues to meet a variety of introductory college science objectives and has dramatically increased student understanding of the scientific method, botany, and plant science at our institution (see the first photo).

In the second week of the 13-week semester of The Personal Plant Project, groups of four students are provided with a different general observation and question connected to plants (see the table). Attached to these questions is a bit of background and a short collection of relevant citations to primary literature (see supplementary materials, part 1). The groups are asked to design an experiment to investigate these questions, meeting



Harvesting chives.

criteria discussed in class, including independent and dependent variables, controls, and replication (avoiding pseudoreplication). Together, the students compose a proposal. In the third week, with the advisement and approval of the instructor, students devise protocols and implement the experiment, planting seeds and applying initial treatments in our greenhouses. Subsequently, students

Personal Plants, an IBI Prize-winning module, offers students connections to taxonomy and systematics, plant chemistry, water relations, and pollination biology.

are expected to collaboratively maintain their group's plants, apply further treatments, and take measurements as laid out in the initial research plan. Infrequently, students' plants fail to grow, but this multisection course ensures that there are multiple approaches to the same question within the class and that students can use one another's data.

Because the outcomes of these experiments take 8+ weeks to manifest, interim assignments throughout the semester are designed to keep the students engaged with their central question and to connect their project to lecture material (see supplementary materials, part 2). These assignments are designed to provide students with information they will eventually incorporate into their final paper. Other shorter inquiry-based and observational labs are conducted to address other plant science topics throughout the term and to supplement the lecture, ranging from designing experiments to explain variation in transpiration rates to manipulating photosynthetic rates in *Elodea*. They also gain experience in writing and rewriting drafts of sections of a scientific paper, based on their experiments and modeled on published literature. By the end of the term, students have amassed a substantial amount of data. They use these data to evaluate the answer to their



Collaboration. Students work together to answer questions about plant growth and productivity.

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*IBI, Science Prize for Inquiry-Based Instruction; www.sciencemag.org/site/feature/data/prizes/inquiry/

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Sample prompts for Personal Plant investigations

Gardeners are often told not to plant carrots and dill next to each other. Why?

Should I invest in bone meal for fertilizing plants at my organic cucumber farm?

My seed packet recommends sowing basil seeds at least 4 to 6 inches apart, but I can get more plants into the garden by planting them closer. Should I ignore the directions?

Farmers often rotate legume and grain plants on the same farmland in successive years. Why?

Is it worth paying extra for potting soil with mycorrhizal fungal spores in it?

initial prompt, which they share with the rest of the class through informal presentations and write a complete scientific paper. This exercise is embedded in a required course in our introductory sequence. The first semester focuses on animals, this term focuses on plants, and the third term on microbes and cells. Students often come to the class with several deep prejudices about plants. They are of the opinion that plants are barely alive, that they are dull and uninteresting, and that they are really not all that important (4, 5). However, the engagement this personal plant project offers substantially changes student attitudes. Students develop personal connections with their plants as they watch them grow from seed to flowering adult—some even get upset when they realize that at least some of their plants must die to allow measurements of final dry biomass.

The research questions framing this personal plant assignment are generally connected to common observations that do not require much special knowledge, although students' lack of exposure to agriculture and gardening make many of these simple descriptive patterns qualify as "revelations."

Because the prompts are fairly simple and investigations of causes for patterns come to mind readily, students are able to focus on applying their understanding of experimental design and the scientific method without having to understand a lot of jargon or terminology. They have opportunities later in the semester to build their own framework for understanding the behavior of individual plant species and to explain the patterns they see. Student findings from the interim assignments are often reintegrated into the final project.

Over the 10 years we have used this project at Rider, we have made many modifications. Although Personal Plants was initially

designed for individual projects, we have found that students prefer to work in groups (see the second photo). Students learn a great deal about the importance of keeping records in the process of keeping a lab notebook, an experience they prefer to using our online learning management system (LMS) for recordkeeping. In two iterations of the course in 2002 and 2003, students were asked to use our LMS to keep blogs about their experiments, but at that point, students were not prepared to apply the technology. Most recently, upper-level bioinformatics students served as statistical consultants for the personal plant student groups. This experience required the Personal Plant Investigators to organize their data in preparation for the consultant visit and offered upper-level students the opportunity to share what they had learned and solve a real problem using real data.

Although most students design experiments that are straightforward, some build complex multifactorial designs and obtain findings that bear repeating or expansion in other courses. For instance, freshmen found that a native plant (evening primrose) grew poorly in soils enriched with activated carbon, a compound often used in experimental settings to absorb allelochemicals produced by plants. In response, students in an advanced botany course constructed an experiment to examine competitive outcomes between evening primrose and a variety of exotic, invasive species in the presence or absence of activated carbon.

Students have independently sought out information on the resources for which plants compete, rhizobia symbioses, allelopathy, or the need for mineral nutrients. Throughout the semester, students share their progress across groups in their lab in informal conversation—devising approaches to reduce insect infestations, teaching each other about the uses of various measurement tools, and ending the semester with both formal written and informal oral summaries of their work and findings.

To assess the extent to which this experience helps students with experimental design, a final exam question asks students to describe a pattern observed during their last lab on a nature walk and to design an experiment to test a hypothetical explanation for that pattern. Students are told during the lab that they should use the time to prepare their answers to this upcoming final exam question. Their responses are graded on the basis of the testability of their hypotheses, the graphs they construct illustrating supportive and refuting results they might find, and the

About the author



Laura A. Hyatt is associate professor of Biology at Rider University where she studies the population biology and chemical ecology of exotic, invasive plant species. She is the founding director of Rider's Sustainability Studies program and currently serves as associate dean in Rider's College of Liberal Arts, Education, and Sciences.

quality of their experimental design. All the criteria for this exam question are embedded in the personal plant assignments throughout the semester. Students have constructed extremely imaginative (and sometimes fantastical) hypotheses and experiments to explain patterns of the distribution of skunk cabbage, mayapples and pussy willows.

Although most students in this class do not go on to a career in the plant sciences, they complete this project with a deeper understanding of plant life, an exposure to basic primary literature, and experience with the methods scientists use in their work. Most of them can also correctly pronounce the Latin binomial of their personal plant 3 years later, at graduation.

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Supplementary Materials

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AAAS S&T POLICY FELLOWS

Four Decades On, Fellows Make Global Impact on Science Policy

The year was 1973, and molecular biologist Jessica Tuchman was preparing to go to work as a congressional staffer, one of seven researchers in the inaugural class of the AAAS Science & Technology Policy Fellows. The timing was auspicious: Energy, nuclear power, and the environment had emerged as urgent issues, and while Congress had just opened its Office of Technology Assessment, scientific expertise remained in profoundly short supply on Capitol Hill.

"There was a need, and there was a demand," she recalls. "This was a moment when Congress really was wrestling with, 'How do we get more advice, more help [on science-related issues]?' It was a wonderful, very fertile moment for us."

Today, Jessica Tuchman Mathews is president of the Carnegie Endowment for International Peace, and her accomplishments during the past four decades reflect the growing impact of the AAAS Fellowships. A total of 2616 scientists and engineers have served as Fellows—including a record 279 in this fall's 40th class—and in a series of interviews, alumni described how the experience has helped to transform both U.S. science policy and their own careers.

After serving 1 or 2 years in nonpartisan congressional and executive branch positions, Fellows have gone on to work "in nonprofits, academic institutions, industry, and, of course, government," said Program Director Cynthia Robinson. "All of those individuals have taken the experience they acquired...in Washington, D.C., and are now applying it in the work they're doing across those sectors—at national, local, and even international levels of policy."

The fourth class, in 1976, had grown to 13 Fellows, including physicist E. William Colglazier. He would go on to become executive officer of the National Academy of Sciences and now S&T adviser to the U.S. Secretary of State. "It's a stroke of genius that this program was created," he said.

How to gauge the Fellowships' impact? Dating back to the earliest classes, alumni can point to specific influence on debates or



Life-changing experience. Jessica Tuchman Mathews is president of the Carnegie Endowment for International Peace. In 1973, she was in the small first class of AAAS Science & Technology Policy Fellows.

legislation—on regulations for intellectual property and biotechnology in the 1980s or, more recently, shaping federal policy for adaptation to climate change, engaging with former Iraqi weapons scientists, and responding to natural disasters in Indonesia and Haiti.

But numbers, too, tell an important part of the story. For example, in 2000, only a few Fellows were assigned to the U.S. State Department. By early 2012, there were 41, and 43 more at the U.S. Agency for International Development (USAID); over 120 former Fellows were employed at State and USAID. Overall, more than 50% continue in government service after their Fellowships end. And the original four science and engineering societies that helped sponsor Fellows have grown to 32.

In an era when science is a key component of so many national and international issues, the Fellowships have "had a very permeating, dramatic effect" on policy-making, said 1985 Diplomacy Fellow Kerri-Ann Jones, the assistant U.S. secretary of state for oceans and international environmental and scientific affairs. "There's this base of scientific wisdom, knowledge, and experience now, throughout the government, that wasn't there before."

1984 Fellow Arati Prabhakar, the director of the Defense Advanced Research Projects Agency (DARPA), offered a related view. "DARPA has had a series of highly successful program managers and

executives, some of whom, if you trace the history all the way back, first came to Washington as Congressional Fellows," she said. "I don't think that's an accident."

The transformative influence of the Fellows works at an individual level, too. Wyoming State Senator Chris Rothfuss, a Diplomacy Fellow from 2003 to 2005, said his experience in Washington is prime subject matter in the classes he teaches in nanotechnology and international relations at the University of Wyoming. Ellen Bergfeld is CEO of the Alliance of Crop, Soil and Environmental Science Societies and related U.S. agricultural organizations—and she credits the experience and contacts derived from her 1996 Congressional Fellowship.

Physicist and U.S. Representative Rush Holt (D–New Jersey), a 1982 Fellow, put it simply: "It was really life-changing... I wouldn't be in Congress now if it hadn't been for the Fellows program."

But alumni emphasize that benefits flow the other way, too. Just as the Fellowships impart scientific expertise to the policy process, they also enrich the research enterprise "by bringing a political savvy back to the professions," Holt said. "And that's an unbeatable combination."

The AAAS Science and Technology Fellowships are planning a series of special events beginning in early 2013, including a speaker series, online workshops, an art exhibit, and a 40th anniversary commemoration.

—Carla Schaffer and Edward W. Lempinen



Scientific Thinking in Young Children: Theoretical Advances, Empirical Research, and Policy Implications

Alison Gopnik

New theoretical ideas and empirical research show that very young children's learning and thinking are strikingly similar to much learning and thinking in science. Preschoolers test hypotheses against data and make causal inferences; they learn from statistics and informal experimentation, and from watching and listening to others. The mathematical framework of probabilistic models and Bayesian inference can describe this learning in precise ways. These discoveries have implications for early childhood education and policy. In particular, they suggest both that early childhood experience is extremely important and that the trend toward more structured and academic early childhood programs is misguided.

Thirty years ago, the idea that 2-year-olds think like scientists would have seemed absurd. Jean Piaget, the great pioneer of cognitive development, claimed that preschoolers' thinking was just the opposite of scientific thinking. Preschoolers were irrational, illogical, "pre-causal," and limited to the here and now (1). These ideas informed both education and policy.

These claims have turned out to be wrong. Several waves of empirical work have shown that even infants and very young children have intuitive theories of the world around them. More recently, mathematical models of learning have been developed. Empirical research informed by those models shows that early learning is also remarkably similar to scientific induction (Fig. 1).

During the 1980s and 1990s, researchers discovered that very young children have abstract, structured, coherent, causal representations of the world around them—representations that are similar to scientific theories. They use those representations to make wide-ranging new predictions. These representations appear to be in place even in infancy, but it is particularly clear that preschoolers have intuitive theories of the physical, biological, psychological, and social world (2–4).

New methods led to this first revolution in our understanding of development. The advent of video recording, and striking experimental ingenuity, led to a flood of results that showed sophisticated knowledge in even the youngest infants. By studying what babies looked at, reached for, or imitated, researchers could show that even infants understand both physical objects and other people [e.g., (3, 5)]. Piaget tried to assess preschool children's knowledge by asking them open-ended questions about hypothetical scenarios. But preschoolers show much more

sophisticated knowledge when they respond to focused questions about real-life examples (2–4).

This research showed that young children's knowledge is structurally similar to scientific theories, but not necessarily that children learn like scientists. It could be that much of this knowledge is innate rather than learned—evolutionarily determined rather than inferred from experience. Moreover, until quite recently, there were few theoretical accounts that could encompass learning mechanisms in both childhood and science, or empirical results that showed those mechanisms were similar. In fact, the predominant theories of learning emphasized complex associations be-

tween stimuli. Associative learning appears to be very different from the hypothesis testing and experimentation of science.

In the past 10 years, however, theoretical and empirical research has begun to show that children's learning mechanisms do indeed resemble the basic inductive processes of science. We now have a more precise and formal theory of children's learning mechanisms, derived from ideas about probabilistic models and Bayesian learning methods that originated in computer science, statistics, and philosophy of science.

Probabilistic Models

Philosophy of science, artificial intelligence, and developmental psychology all face the same fundamental dilemma. As adults, we seem to have highly structured, abstract, coherent knowledge of the world around us. This knowledge allows us to make wide-ranging predictions and inferences. But we also seem to learn that highly structured knowledge from the contingent, concrete, probabilistic evidence of our senses. How can this be? Traditionally, philosophers and psychologists have responded to this dilemma in two ways. "Nativists" have argued that this abstract structure must be in place innately because it could not possibly be learned. "Empiricists" have argued that this abstract structure is illusory; in reality there are only specific learned associations between particular pieces of evidence.

The probabilistic models approach [e.g., (6–9)] addresses this dilemma in a new way. Imagine that there is some real structure in the world—a spatial configuration, a grammar, or a network of causal relationships. That structure gives rise



Fig. 1. Child's play is science. ["Playing Doctors" by Frederick Daniel Hardy (1827–1911); image: Stapleton Collection/Corbis]

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to some patterns of evidence rather than others—a particular set of visual images, or spoken sentences, or statistical contingencies between events. That spatial or grammatical or causal structure can be represented mathematically by a generative model, such as a map or tree structure or a graphical network. The representation is a hypothesis about what the actual structure is like. This hypothesis can be precisely described in formal ways. The representation is generative, which means that it will allow you to mathematically compute the patterns of evidence that follow from that structure and then make new inferences accordingly: A particular map will let you predict how to reach a location by a new route; a particular grammatical tree will let you predict whether a new sentence will be acceptable; a particular causal graph will let you predict whether a new event will be followed by other events. If the hypothesis is correct, then these predictions will turn out to be right.

These generative models, then, can describe representations of the world and explain how those representations allow us to make a wide range of new inferences. Critically, the systematic link between structure and evidence in these models also allows you to reverse the process and to make inferences about the nature of the structure from the evidence it generates. It lets you decide which map or tree or causal graph best accounts for the evidence, and so leads you to adopt the most likely hypothesis.

The idea that mental models of the structure of the world generate predictions, and that we can invert that process to learn the structure from evidence, is not itself new. The big advance has been integrating ideas about probability into that basic framework. Typically, a great many hypotheses are, in principle, compatible with any pattern of evidence, so how can we decide on the best one? Integrating probability theory makes this learning problem more tractable. Although many hypotheses may be compatible with the evidence, some hypotheses will be more or less likely to have generated the evidence than others.

One of the most powerful and general ways to solve the learning problem is to use Bayesian inference. If we know the prior probability of a hypothesis, and a generative model tells us the likelihood of the evidence given the hypothesis, then when we observe a new pattern of evidence, we can use Bayes' rule to determine the probability that the hypothesis is true given that evidence. Rather than simply generating a yes-or-no decision about whether a particular hypothesis is true, the probabilistic Bayesian learning algorithms consider multiple hypotheses and assign probabilities to those hypotheses. Bayesian methods let you determine the probability of possibilities.

Bayesian ideas have been successfully applied to a wide range of problems, including vision and motor control (10, 11). This kind of perceptual and motor learning may not appear to resemble scientific learning. But probabilistic models have also been applied to precisely the kinds of knowl-

edge that we see in scientific and intuitive theories. In particular, causal knowledge is central to both kinds of theories. Causal graphical models or "Bayes nets," developed in the philosophy of science and computer science, provide a particularly powerful and successful account of causal knowledge and learning (6, 12, 13). Algorithms that use Bayes nets allow computers to actually do some kinds of science, such as discovering the causal structure of weather systems, gene expression, or brain function from data.

Most recently, this work has been expanded to allow for formal representations of more abstract

higher-order causal structure—for example, the general framework principles that prevail in a scientific paradigm and that shape particular causal hypotheses (14). There has also been work on an even more general "probabilistic logic" that can encode a much wider range of relationships, including spatial and logical as well as causal ones. At least in principle, this logic allows a wide range of generative models to be learned from probabilistic data (15).

Unlike traditional nativism, the probabilistic models approach gives us a way to actually infer abstract hierarchical structure from data, at least

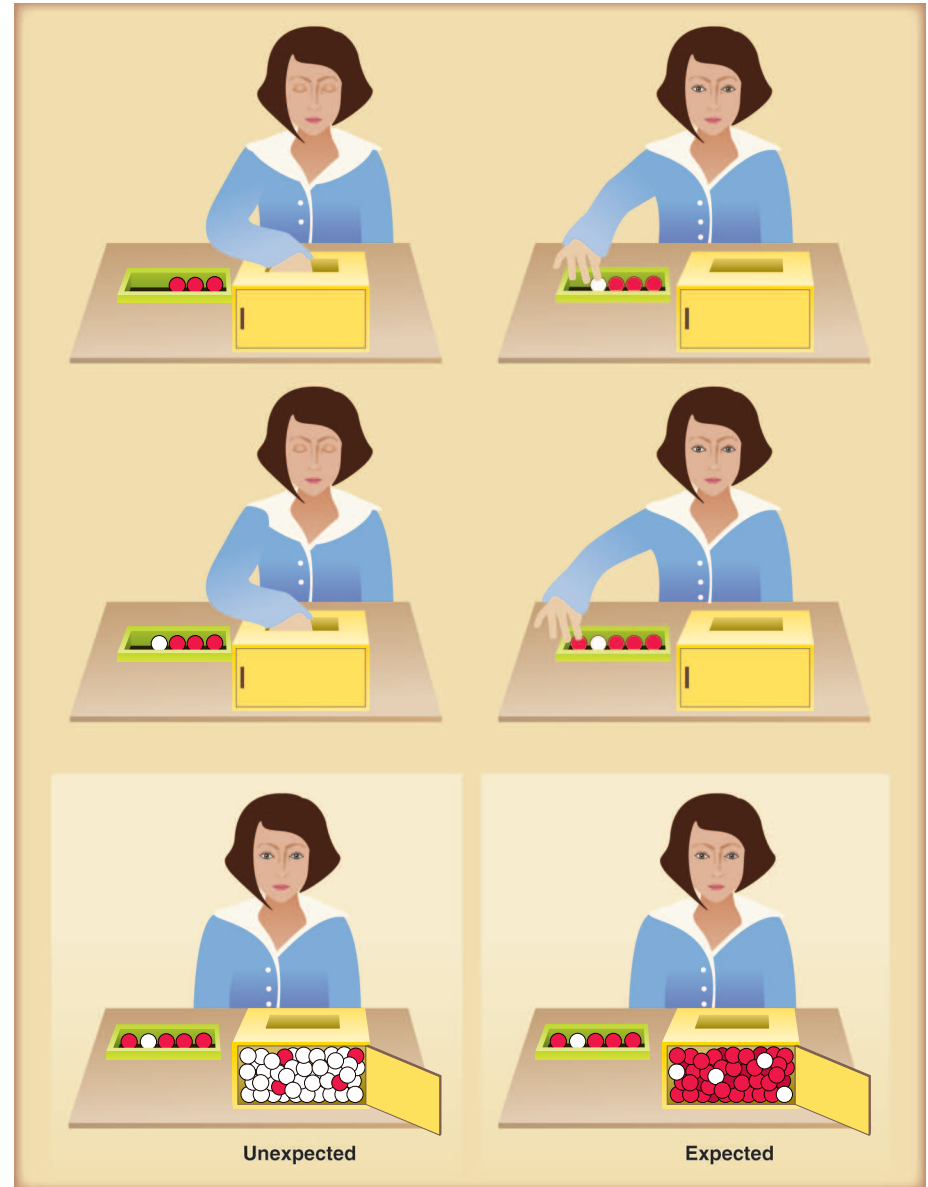


Fig. 2. Schematic representation of the ping-pong ball experiment. The experimenter showed the infants a box full of white and red balls. Then she closed her eyes and randomly took some balls from the box and put them in another small bin. If the sample was truly random, then the distribution of balls in the bin should match the distribution of the balls in the box. Infants saw a sample that either matched or did not match the distribution, and they looked longer at the nonmatching sample. In a control condition, infants saw just the same sequence of events, but the experimenter took the balls out of her pocket rather than taking them from the box, and the looking-time difference disappeared.

in principle. If children learned in this way, they could drastically revise their representations of the world on the basis of their experience, as scientists do. They would not be limited to making small adjustments to innately determined representations. Unlike traditional empiricism, the approach proposes that children, also like scientists, never start from a completely blank slate or with completely pure data. Instead, from the very beginning, they would be testing hypotheses and assessing the data in the light of those hypotheses.

Children as Scientific Learners

Do children actually learn about the world in this way? Over the past 10 years, researchers have systematically given young children patterns of evidence about the world and then observed the conclusions that they draw [e.g., (7) and articles in (16, 17); for an extensive review and tutorial, see (18)]. To a striking extent, children use data to formulate and test hypotheses and theories in much the same way that scientists do. Scientists learn about the world in three ways: They analyze statistical patterns in the data, they do experiments, and they learn from the data and ideas of other scientists. The recent studies show that children also learn in these ways and that they often resemble ideal Bayesian learners. Probabilistic models make accurate and detailed predictions about children's learning.

Statistics. Anyone who has ever taught a methods course knows that adults have a hard time

explicitly understanding statistics. It may be surprising, then, that even very young infants can implicitly reason statistically. The first wave of these experiments showed that even young infants are sensitive to statistical patterns [e.g., (19)]. More recently, researchers have shown that infants and young children not only detect statistical patterns, they use those patterns to test causal hypotheses about people and things.

For example, Xu and Garcia (20) demonstrated that 8-month-olds were sensitive to statistical sampling patterns. They used a "looking-time" technique that has been extensively used to study infant cognition. It depends on the fact that infants look longer at unexpected events. When the experimenter took a sample of mostly red ping-pong balls from a box of mostly white balls, infants looked longer than when she took a sample of mostly red balls from a box of mostly red balls (Fig. 2).

Note that the unlikely events in this experiment were not impossible; you could, after all, pull mostly red balls from a box of mostly white balls. The events were merely improbable if your causal model of the event assumed that the balls in the bin were a random sample. It's as if the infants said to themselves, "Aha! Less than 0.05 probability that this occurred by chance!" But would the surprising evidence drive the children to a new causal model?

Kushnir *et al.* (21) found that it would. In fact, children as young as 20 months interpreted non-

random sampling psychologically. An experimenter took frogs from a box of all frogs or she took frogs from a box of almost all ducks. Then she left the room and another experimenter gave the child a small bowl of frogs and a separate bowl of ducks. When the original experimenter returned, she extended her hand ambiguously between the bowls. The children could give her either a frog or a duck. When she had taken frogs from a box of all frogs, children were equally likely to give her a frog or a duck. When she had taken frogs out of the box that was almost all ducks, children gave her a frog. In the first case, the children concluded that she had merely drawn a random sample from the box, but in the second case they concluded that she had displayed a preference for frogs. Thus, children less than 2 years old had inferred an underlying mental state—a preference—from a statistical pattern.

In another line of research, my colleagues and I designed a simple test to see whether young children would appropriately infer physical causal relationships from statistical evidence about covariation (7, 22). We showed children a "blicket detector"—a box that plays music when you put some objects on it but not others—and then showed them various patterns of statistical dependence between the objects and the effect. Then we asked children to make the machine go or turn it off. Figure 3 shows one such experiment. Based on the child's prior knowledge about the machine, it could have any of the causal structures represented in Fig. 3. We found that 2-, 3-, and 4-year-olds could use the pattern of covariation between the blocks and the machine's activation to infer which of these causal structures was correct, and so to make the machine go or stop. Other studies show that toddlers as young as 24 months can make these inferences even when the statistical pattern is more complicated (23). One recent study shows that even 16-month-olds can use covariation to infer causation in this way (24).

Schulz *et al.* (25) showed that 4-year-old children could also use statistical dependencies to infer more complex causal structures. Children saw a simple machine with a switch on one side and two disks that spun on top. Even this simple machine could work in many different ways (the switch could make the blue disk go, which could make the yellow disk go; the switch could make both disks go; etc.). Preschoolers used evidence correctly to distinguish between causal chain structures (the switch makes the blue disk go, which makes the yellow disk go), common cause structures (the switch makes both disks go), and conjunctive cause structures (the switch and the blue disk are both necessary to make the yellow disk go).

Bayesian inference considers both new evidence and the prior probability of hypotheses. This gives Bayesian learning a characteristic combination of stability and flexibility. In science, we hold on to well-confirmed hypotheses, but enough new evidence can eventually overturn even the

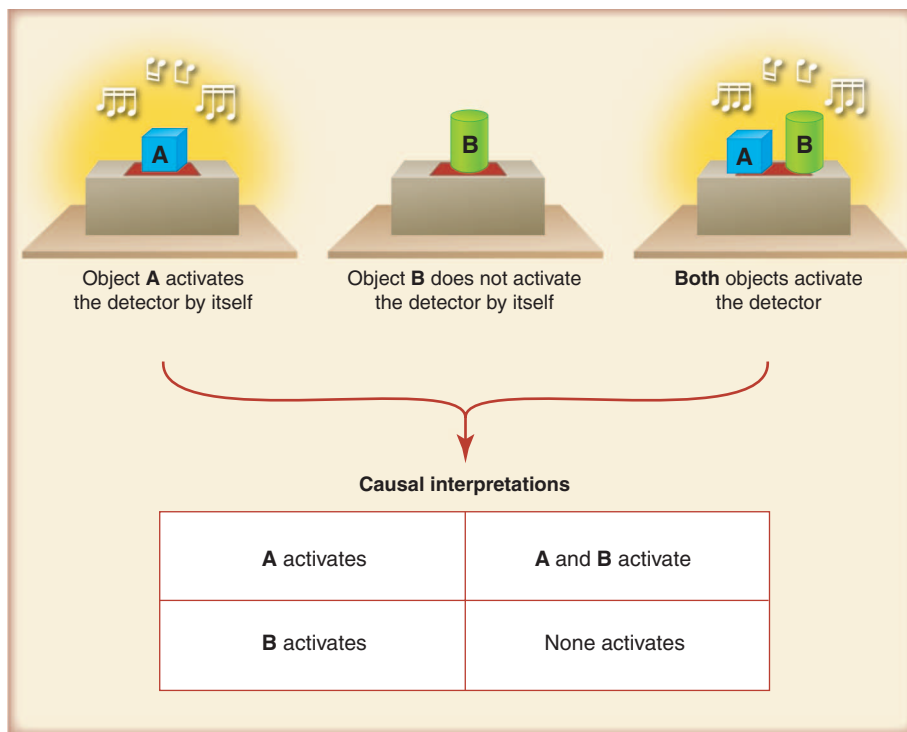


Fig. 3. The blicket detector experiment. Children saw that the machine did not activate when B alone was placed on it, but did activate when A was placed on it and continued to do so when B was added to A. Then they were asked to make the machine stop. Given this evidence, the correct causal interpretation is that A alone activates the machine, and the children should act on A and not B.

most cherished idea. Several recent studies show that children integrate prior knowledge and new evidence, too. For example, 4-year-olds begin by thinking that psychological causes (e.g., being anxious) are unlikely to cause physical effects (e.g., having a stomach ache) and reject evidence to the contrary. But if you give them accumulating evidence in favor of this “psychosomatic” hypothesis, they gradually become more and more likely to accept that initially unlikely idea (26), and a Bayesian model can predict this change quite precisely.

Children also use statistics to infer the existence of unobserved causes—hidden “theoretical entities.” Gopnik *et al.* (7) found that when the observed variables couldn’t explain the evidence, children would look for unobserved variables instead, in a way that could be predicted by Bayes nets. Schulz and Sommerville (27) found that when children saw a “blicket detector” that went off only 2 of 6 times, they inferred that some hidden variable was responsible for the failures.

Finally, we can ask whether children are restricted to making specific inferences about particular causal relationships or whether, like scientists, they can also make inferences about broader “framework principles”—general theoretical ideas or “paradigms.” An exciting development in the computational world has been the discovery that more abstract theoretical laws can actually sometimes be learned more quickly than the specific causal hypotheses they subsume (28). Two recent studies also suggest that preschoolers can make these broader generalizations swiftly and appropriately (29, 30).

Experiments. Anyone who watches young children has seen how they ceaselessly fiddle with things and observe the results. Children’s play can look like experimentation. Recent research by Schulz and colleagues shows that children’s exploratory play does indeed involve a kind of intuitive experimentation. Children’s play is not as structured as the ideal experiments of institutional science; even adults can have a hard time designing ideally controlled experiments [e.g., (31)]. However, recent formal work in the philosophy of science has shown that much less systematic experimentation can yield a remarkable amount of causal knowledge (32). The empirical research shows that play is sufficiently systematic to help children discover causal relationships.

For example, Cook *et al.* (33) performed a variant of the “blicket detector” experiments using “pop-beads,” small plastic beads that could be hooked together to make larger units. First, the experimenter put individual beads on the machine. One group of 4-year-olds saw that some of the beads made the machine go and some didn’t. A second group saw that all the beads made the machine go. Then, the experimenter simply gave the children the machine and two new beads that were hooked together and let them play.

The “some beads” condition sets up a causal problem for the children: Which beads make the machine go? To solve that problem, you need to

test each bead by itself. The “all beads” condition does not; children can assume that both beads will make the machine go. Sure enough, children spontaneously pulled the beads apart and tested them separately in their play in the “some beads” condition but not in the otherwise identical “all beads” condition.

Similarly, Legare (34) showed 4-year-olds that red blocks made a “blicket detector” machine go and then showed them an anomaly—a red block that failed. She asked them “Why did that happen?” and let them play with the machine. Children systematically played with the machine in ways that tested the hypotheses that they expressed in their explanations. Another recent study shows that pretend play is also closely related to counterfactual reasoning—a particularly sophisticated type of causal inference (35).

These results indicate that when young children face a causal puzzle, they try to solve that puzzle in their spontaneous play. Children’s actions ensure that they receive causally relevant and informative evidence. Once that evidence is generated through play, children can use it to make the correct causal inferences.

Learning from Others

The picture of the child as a “little scientist” has sometimes been taken to imply that children are solitary learners. Of course, real science is a highly social endeavor, and scientists must constantly interpret the demonstrations and reports of other people. Children can also learn about causal relationships by watching what other people do and what happens as a result. In our lab (36), 4-year-olds saw an experimenter perform five different sequences of three actions on a toy, which activated or did not activate on each trial. A statistical analysis of the data would suggest that only the last two actions were necessary to activate the toy. When children got the toy, they often produced just the two relevant actions, rather than imitating everything that the experimenter did.

Moreover, very young children and even infants are sensitive to the intentions of others, particularly their intention to teach, and may draw different conclusions from the evidence that teachers give them than from the evidence they gather themselves. Bayesian models can incorporate pedagogical information by assuming that teachers provide a different and more informative sample of evidence than one would get from a random sample (37).

We (36) did exactly the same statistical imitation experiment but now included pedagogical information: The experimenter said “Here’s my toy, I’m going to show you how it works.” In this condition, children were much more likely to assume that everything the adult did was causally effective and to imitate all her actions. A Bayesian model made quite precise quantitative predictions about what the children would do in the pedagogical and nonpedagogical context.

Similarly, Bonawitz *et al.* (38) gave children a complicated toy to explore. The toy had four

tubes, each of which did something different (one lit up, one made a squeaking sound, etc.). In one condition, children saw the experimenter accidentally bump against the toy, setting off one of the squeaky tubes. Then she simply left the child alone to play with the toy. The children imitated the squeak but also discovered all the other things that the toy could do. In another condition, the experimenter introduced the toy by saying “Here is my toy” and then made it squeak. Like the children in the imitation experiment, children in this condition simply repeated what the experimenter did, and didn’t explore the machine’s other possibilities.

These new studies, and many similar ones, suggest that children as well as scientists learn in ways that are well described by probabilistic models. This research also raises myriad new problems and exciting directions for further work. How are these abstract computations actually implemented in detail by limited human minds and, ultimately, how are they instantiated in human brains? Children and scientists often seem to develop radically new hypotheses. Where do these hypotheses come from? Are children simply learners with less experience—in Bayesian terms, do they simply have a different prior? Or do they learn in ways that are Bayesian but are qualitatively different from adult learning? For example, do they search a wider space of hypotheses than adults do (29)? How is this learning influenced by the development of explicit symbol systems, from language itself to the complex mathematical notation of physics? What is the ideal balance between individual discovery and learning from others, and does this balance shift in different educational and scientific contexts? The new theoretical ideas and experimental methods give us a framework for asking and answering these questions.

Implications for Policy

So new theoretical work lets us describe both scientific learning and children’s learning in a newly systematic and rigorous way. New empirical work shows that young children learn from statistics, experiments (i.e., play) and from the actions of others in much the same way that scientists do. What does all this mean for education and policy?

First, this work provides an explanatory foundation for the demonstrable impact of high-quality preschool and care for children on later life (39). Very young children are spontaneously and pervasively learning from experience. Moreover, the Bayesian picture provides a better model for these effects than thinking about early childhood as an irreversible “critical period.” Without the right sequence of evidence, theoretical advances will be delayed or may never emerge at all. Conversely, when particular hypotheses are especially well confirmed early in life (hypotheses, for example, that expressing distress causes caregivers to turn away, or that threat leads to violence), it may be much more difficult for them to be revised later on. Even the most entrenched dysfunctional

models can, however, be overturned with sufficient evidence.

This work also should raise serious red flags about recent pressure, both from parents and policy-makers, to make preschools more structured and academic—more like schools. This research is new, so it's not surprising that early childhood policy-makers still routinely hold an outdated view of development. According to this view, early childhood is about "socioemotional" development, in contrast to "cognitive skills," which are identified with later scholastic abilities such as reading and calculating. Policy-makers may acknowledge the importance of the "socioemotional" aspect, but they systematically underestimate the intellectual capabilities of preschoolers. The new research shows that even very young children are deeply engaged in such profoundly cognitive work as hypothesis testing and causal inference. This work is more cognitively challenging, in fact, than much school work.

Moreover, the research has begun to demonstrate scientifically what most preschool teachers feel intuitively. Children's spontaneous exploratory and pretend play is designed to help them learn. And pedagogy can be a mixed blessing. Even preschoolers know when they are being taught, and quickly take on information from teachers. But explicit teaching can also narrow the range of hypotheses that children are willing to consider. Activities such as encouraging play, presenting anomalies, and asking for explanations prompt scientific thinking more effectively than direct instruction.

Finally, the new research suggests that our everyday thinking and learning is strikingly continuous with scientific thinking and learning. Of course, formal scientific thinking involves a level of self-conscious reflection, including reflection on the very process of science itself. We don't see this reflection in very young children: The preschoolers see probabilistic evidence and revise hypotheses, but they don't necessarily know that that is what they are doing—nor indeed do ordinary adults. Nonetheless, we should be able to exploit the fact that very young children are natural scientists in action to help them under-

stand the principles of formal science. Even university students can understand statistics much more effectively when the material is presented in the context of everyday causal inference (40). Ordinary adults might also learn scientific concepts more effectively through play, experimentation, and observation than through pedagogy.

The new work, then, provides a scientific foundation for a long tradition of "inquiry-based" science education. But our new understanding of children's intuitive science ought to help us go beyond just a general emphasis on active inquiry. Instead, it could lead us to much more specific and scientifically supported proposals for education. Science itself could help turn young children's natural curiosity and brilliance into better science teaching and learning.

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Next-Generation Digital Information Storage in DNA

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As digital information continues to accumulate, higher density and longer-term storage solutions are necessary (1). DNA has many potential advantages as a medium for immutable, high latency information storage needs (2). For example, DNA storage is very dense. At theoretical maximum, DNA can encode two bits per nucleotide (nt) or 455 exabytes per gram of single-stranded DNA (3). Unlike most digital storage media, DNA storage is not restricted to a planar layer and is often readable despite degradation in nonideal conditions over millennia (4, 5). Lastly, DNA's essential biological role provides access to natural reading and writing enzymes and ensures that DNA will remain a readable standard for the foreseeable future.

Storing messages in DNA was first demonstrated in 1988 (6), and the largest project to date encoded 7920 bits (7). The small scale of previous work stems from the difficulty of writing and reading long perfect DNA sequences and has limited broader applications (table S1). We developed a strategy to encode arbitrary digital information by using a novel encoding scheme that uses next-generation DNA synthesis and sequencing technologies (fig. S1). We converted an html-coded draft of a book that included 53,426 words, 11 JPG images, and one JavaScript program into a 5.27-megabit bitstream (3). We then encoded these bits onto 54,898 159-nt oligonucleotides (oligos) each encoding a 96-bit data block (96 nt), a 19-bit address specifying the location of the data block in the bit stream (19 nt), and flanking 22-nt common sequences for amplification and sequencing. The oligo library was synthesized by ink-jet printed, high-fidelity DNA microchips (8). To read the encoded book, we amplified the library by limited-cycle polymerase chain reaction and then sequenced on a single lane of an Illumina HiSeq. We joined overlapping paired-end 100-nt reads to reduce the effect of sequencing error (9). Then with only reads that gave the expected 115-nt length and perfect barcode sequences, we generated consensus at each base of each data block at an average of ~3000-fold coverage (fig S2). All data blocks were recovered with a total of 10 bit errors out of 5.27 million (table S2), which were predominantly located within homopolymer runs at the end of the oligo, where we only had single sequence coverage (3).

Our method has at least five advantages over past DNA storage approaches. We encode one bit per base (A or C for zero, G or T for one), instead of two. This allows us to encode messages many ways in order to avoid sequences that are difficult

to read or write such as extreme GC content, repeats, or secondary structure. By splitting the bit stream into addressed data blocks, we eliminate the need for long DNA constructs that are difficult to assemble at this scale. To avoid cloning and sequence verifying constructs, we synthesized, stored, and sequenced many copies of each individual oligo. Because errors in synthesis and sequencing are rarely coincident, each molecular copy corrects errors in the other copies. We used a purely in vitro approach that avoids cloning and stability issues of in vivo approaches. Lastly, we leveraged next-generation technologies in both DNA synthesis and sequencing to allow for encoding and decoding of large amounts of information for ~100,000-fold less cost than first-generation encodings.

The density (5.5 petabits/mm³ at 100× synthetic coverage) and scale (5.27 megabits) of this work compare favorably to other experimental storage technologies while only using commercially available materials and instruments (Fig. 1 and table S3). DNA is particularly suitable for immutable, high-latency, sequential access applications such as archival storage. Density, stability, and energy efficiency are all potential advantages of DNA storage (10), although costs and times for writing and reading are currently impractical for all but century-scale archives (3). However, the costs of DNA synthesis and sequencing have

been dropping at exponential rates of 5- and 12-fold per year, respectively—much faster than electronic media at 1.6-fold per year (11). Handheld, single-molecule DNA sequencers are becoming available and would vastly simplify reading DNA-encoded information (12). Our general approach of using addressed data blocks combined with library synthesis and consensus sequencing should be compatible with future DNA sequencing and synthesis technologies. Reciprocally, large-scale use of DNA such as for information storage could accelerate development of synthesis and sequencing technologies (13). Future work could use compression, redundant encodings, parity checks, and error correction to improve density, error rate, and safety. Other polymers or DNA modifications can also be considered to maximize reading, writing, and storage capabilities (14).

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Supplementary Materials

www.sciencemag.org/content/full/science.1226355/DC1
Materials and Methods
Supplementary Text
Figs. S1 and S2
Tables S1 to S3
References (15–35)

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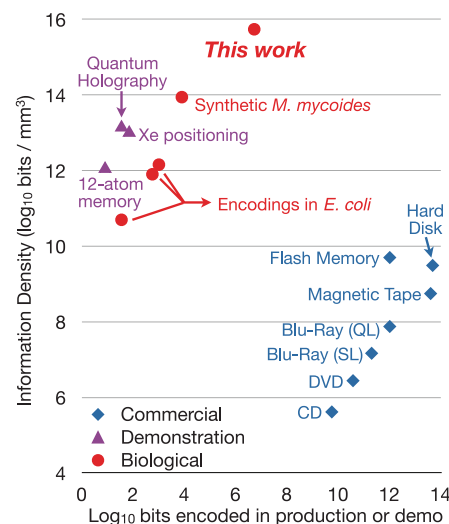


Fig. 1. Comparison to other technologies. We plotted information density ($\log_{10}$ of bits/mm³) versus current scalability as measured by the $\log_{10}$ of bits encoded in the report or commercial unit (3).

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Out of the Tropics: The Pacific, Great Basin Lakes, and Late Pleistocene Water Cycle in the Western United States

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The water cycle in the western United States changed dramatically over glacial cycles. In the past 20,000 years, higher precipitation caused desert lakes to form which have since dried out. Higher glacial precipitation has been hypothesized to result from a southward shift of Pacific winter storm tracks. We compared Pacific Ocean data to lake levels from the interior west and found that Great Basin lake high stands are older than coastal wet periods at the same latitude. Westerly storms were not the source of high precipitation. Instead, air masses from the tropical Pacific were transported northward, bringing more precipitation into the Great Basin when coastal California was still dry. The changing climate during the deglaciation altered precipitation source regions and strongly affected the regional water cycle.

The Great Basin and southwestern United States were wetter during the last glacial period than today. From Oregon to West Texas, many modern desert valleys were once extensive lakes or wetlands. Largest and best known are Lake Bonneville, whose remnant is the Great Salt Lake, and Lake Lahontan in Nevada. Many Great Basin valleys have multiple ancient shorelines on valley slopes and exposed subaqueous sedimentary structures that together indicate a complex history of changes in precipitation-evaporation.

After radiocarbon dating became established, lake elevations were used to construct a regional late-Pleistocene climate history (1) for Lakes Lahontan and Bonneville and for many of the other Pleistocene lakes in the western United States (supplementary text). Lake level reconstructions combined with modeling and paleotemperatures could then be used to study how the water cycle changed. Lake levels in closed Great Basin drainages reflect the net water balance between precipitation and temperature-driven evaporation from the lake's surface. The high lake levels of Lake Lahontan were mostly caused by higher precipitation and runoff, with lesser change caused by cooler temperatures (2, 3). The change in evaporation associated with 7°C cooling was sufficient to raise Lake Lahontan level by ~30 m out of the 180-m peak highstand.

Higher precipitation (~140%) and runoff (~280%) are needed to cause peak lake levels. Once formed, the lakes themselves helped to maintain higher water levels by being a source of precipitation to their own watershed (4).

There was major water cycle variability during the deglaciation in the Great Basin (5). A major dry interval between 17.5 and 16 thousand years ago (ka) is proposed to separate an early wet interval from a major wet interval between ~16 and 14.5 ka (5). Our study examines both the timing and geographic distribution of wet intervals to determine the linkage between the Pacific Ocean sources of atmospheric water and the change in the water cycle.

New insights about the water cycle are possible because of a decade's worth of study of sediments from drill sites along the California margin (Fig. 1) (6, 7). Sea surface temperature (SST) reconstructions and pollen data from multiple marine localities record changes in both coastal climate conditions and changes in the North Pacific subtropical gyre (8). Comparisons between climates in the coastal region and in the Great Basin give a geographic perspective about how the water cycle has changed during this major climate change.

The "shift of the westerlies" hypothesis. It has been proposed that elevated lake levels within the Great Basin mark southward shifts of Pacific winter storm tracks during glacials, causing higher regional precipitation (9). According to this hypothesis, the jet stream split because of Cordilleran-Laurentide Ice Sheets, with a weak branch going north of the ice sheet and a strong branch going across southern California. The southern jet carried storms inland to fill the Great Basin lakes (9).

The modeling that helped define this hypothesis has shortcomings. The resolution of the 1980s vintage CCM0 model (9) is 4.4° in latitude and

7.5° in longitude, so shifts in storm tracks are not well constrained. SST was based on the early Climate: Long range Investigation, Mapping, and Prediction (CLIMAP) last glacial maximum (LGM) reconstruction (10). The SST fields during deglaciation were not based on data but were interpolated between the LGM CLIMAP SST (10) and modern SST. New high-resolution model results based on the LGM CLIMAP SST (10) show LGM results similar to Cooperative Holocene Mapping Project (COHMAP), but with important differences (9). The new studies have higher numbers of Pacific storms than do modern conditions such as those of COHMAP (9), but with a weak "southern" winter jet stream and storm tracks that tend to be diverted northward into the Alaska Gyre (11, 12). New multiproxy LGM SST reconstructions have identified errors in the CLIMAP SST reconstruction, which may cause further revisions to LGM climate patterns (13). In the northeast Pacific, alkenone estimates of LGM SST along the California margin (Fig. 1) delineate an 800-km southward shift of the 8°C isotherm compared with CLIMAP. CLIMAP tropical SST appears to be too warm compared with the Multiproxy Approach for the Reconstruction of the Glacial Ocean Surface (MARGO) reconstruction (13). When cooler LGM tropical SST is input to the National Center for Atmospheric Research (NCAR) CCM3 model, temperate Pacific storm energy is weakened compared with simulations that use CLIMAP-based SST (14).

Because Pacific SST maps for each of the 3-thousand-year (ky) time slices in the COHMAP (9) reconstruction are interpolations, better information is needed to understand the deglaciation in the western United States. Better lake hydrographs in the Great Basin and better information about both eastern Pacific SST (8, 15–20) and coastal vegetation (8, 21–25) sharpen the temporal and spatial pattern of wet intervals to compare with the model of shifting westerlies. The new data suggest that a strong tropical connection at the LGM and during the deglaciation was important to western U.S. precipitation, rather than southward shifts of North Pacific storms.

Pleistocene wet intervals along the U.S. Pacific margin. Timing of wet periods for coastal California are based on the pollen time series. Distinctive pollen assemblages in coastal cores are associated with changes in temperature and/or precipitation in coastal river drainages and can be correlated directly to marine variability (8, 26). As climate cooled, cool temperate coastal forests typically expanded southward. Higher relative precipitation typically causes an increase in forest cover relative to grassland or shrubland, or an increase in wet-adapted versus dry-adapted tree varieties (21, 27). Southern California near Santa Barbara, for example, remained arid throughout the past 160 ky, but wetter intervals are marked by expansion of an upland pine forest (Fig. 2) (21, 28). In central California, decreases in pollen from herbs and shrubs and increases in tree

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pollen—alder, coastal redwood, and oak—mark wet intervals (8). In northern California, both temperature and precipitation affected the record. Alder, oak, and coastal redwood retreated south-

ward at the expense of cool-adapted Sitka spruce and western hemlock as temperatures cooled (22, 23). We used the peak in alder pollen found during the deglaciation to define maximum pre-

cipitation because alder responds rapidly to changing conditions and was well within its temperature tolerance, even at the glacial maximum (19).

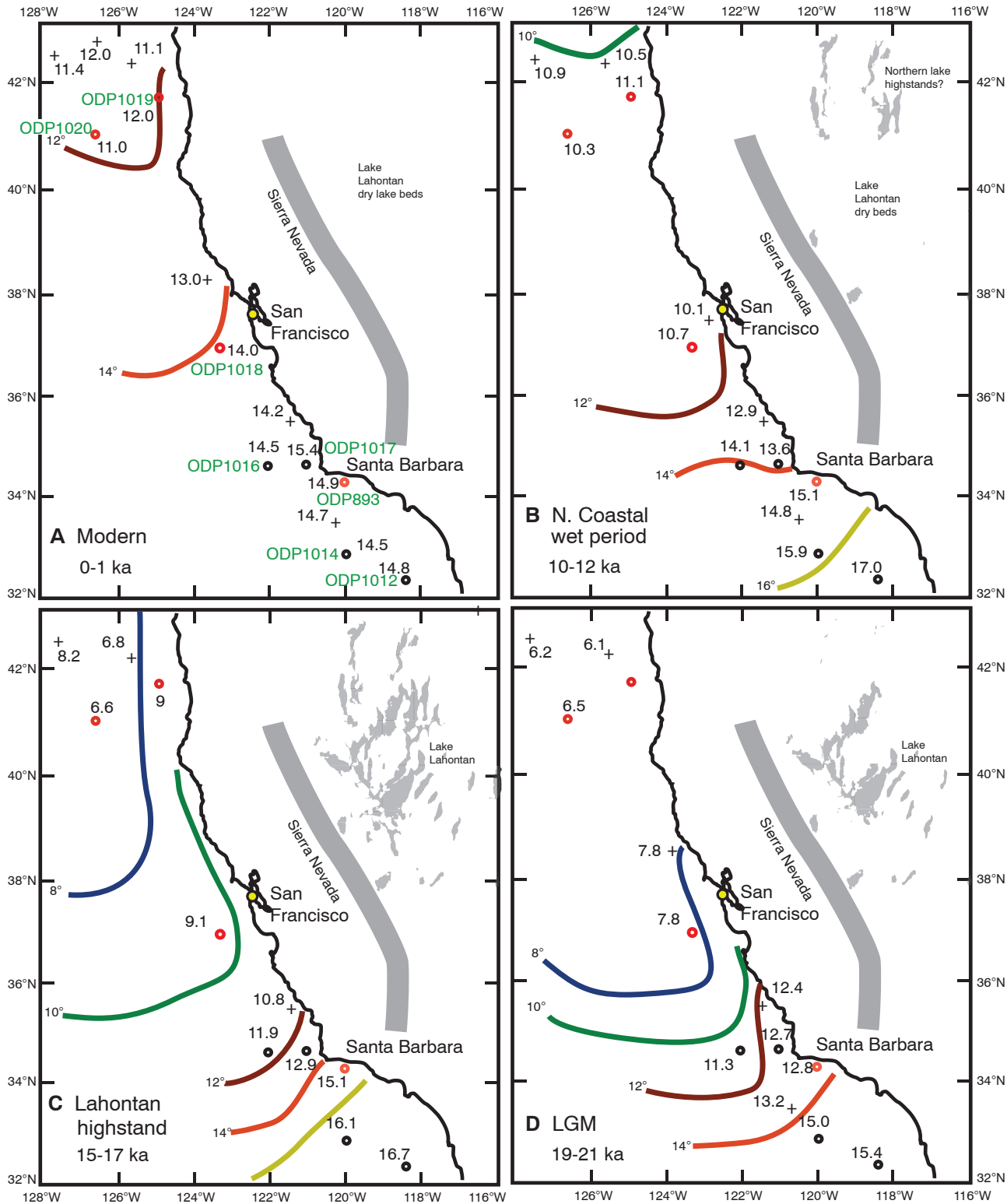


Fig. 1. Alkenone estimates of mean annual SST for California margin time slices (8, 15–20, 39), plus one radiolarian SST estimate at Site 1019 for the 15- to 17-ka time slice (22). (A) Modern era, 0 to 1 ka. (B) North Coastal wet period, 10 to 12 ka. (C) Lahontan highstand, 15 to 17 ka. (D) LGM, 19 to 21 ka.

Sites marked with red open circles also have pollen assemblage data. Sites marked with plus signs are from gravity and piston cores (16); other data are from ODP drill sites, as marked in (A). Warmest SST off southern California and strongest coastal SST gradient occurred in the 15- to 17-ka period.

The southern California wet period [Ocean Drilling Program (ODP) Site 893, 34.3°N] occurs ~5 ky earlier than those in central and northern California (Fig. 2). Maximum wet conditions occurred between 17.4 and 14.2 ka, with much of the glacial interval wetter than modern conditions (21, 24). Central California (ODP Site 1018, 37°N) had a wet interval that was confined to the interval between 12.5 and 4.5 ka, ~5 ky after the Santa Barbara wet interval (8). Similar timing was found in a speleothem record from Moaning Cave at ~38°N in the western foothills of the Sierra Nevada (29). Brief wet intervals were

found at about 14.1 and 13.3 ka, but an extended wet interval began ~12.5 ka and lasted until 9.4 ka.

In northern California (ODP Site 1019, 41.7°N), the wet interval began ~14 ka, peaked ~12 ka—coincident with rapid SST warming along the California-Oregon margin (15, 19)—and ended ~10 ka (19), slightly earlier than the wet interval in central California. The beginning of the wet interval is marked by an abrupt switch from dry, cool conditions inferred by high levels of artemisia and pine pollen to wetter conditions inferred by high levels of alder pollen.

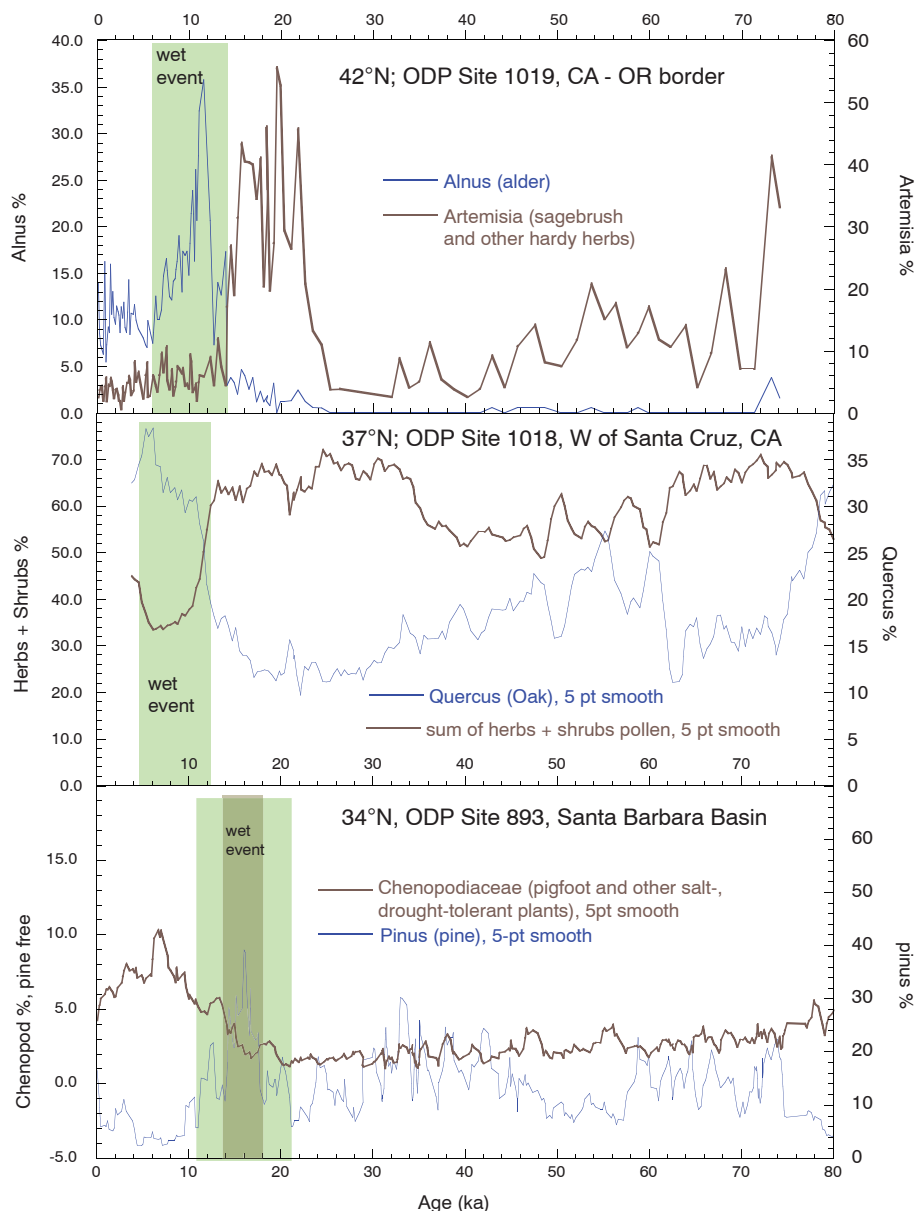


Fig. 2. Pollen records from ODP sites along the California margin used to measure SST and change in vegetation. Green bands denote coastal wet periods and show the early wet period in the south. The blue time series is of pollen from more wet-adapted vegetation, and brown is that from dry-adapted vegetation. (Top) ODP Site 1019, near Crescent City California (42°N) joining two age models: top 16 ka (19), and 16 to 75 ka (40). (Middle) Site 1018, west of Santa Cruz (37°N) (8). (Bottom) Site 893 in the Santa Barbara Basin (34°N) (24).

Timing of lake level change in the Great Basin. If westerly storm tracks first moved southward then subsequently returned to the north, latitude bands should have the same timing for the coastal wet intervals and lake level highstands. By this hypothesis, as the westerlies shifted southward, there should have been pre-LGM drying in the northern Great Basin >40° N, concurrent with high lake levels south of 35° N. As glaciers waned, storms should have swept northward, briefly causing coastal wet intervals and Great Basin lakes in the central latitudes to be high.

We group high lake stands into three major intervals since the LGM: >17 ka, 14 to 17 ka, and <14 ka (Figs. 3 and 4 and table S1). Highest highstands in the 17- to 14-ka range are characteristic of the central Great Basin from ~37°N to 42°N, including Lakes Lahontan and Bonneville. The southern Great Basin, south of 37°N, typically had early lake highstands before 17 ka, or roughly similar highstands in the periods between 17 to 14 ka and >17 ka. The northern Great Basin, north of 42°N, had early maximum highstands ~21 ka (30) but with additional highstands ~13 to 10 ka. Cold temperatures, especially ice formation, may have been the primary factor for older lake highstands in the northern Great Basin, with moderately higher precipitation being a secondary factor (30).

Tests of the westerly storm track hypothesis.

There is little observational evidence to indicate that westerly storm tracks shifted southward at the LGM. First, the timing of wet periods is not the same along latitudinal bands. The highest lake levels in Lakes Bonneville and Lahontan (between 37° and 42°N) occurred between 17 and 14.5 ka, but in the coastal region west of the Sierra and north of 36°N, there were no wet periods then (Fig. 4). Vegetation associated with higher precipitation at Sites 1018 and 1019 (37° and 41.7°N) appears after 12.5 ka (Fig. 2) (8, 19, 22). Only the wet period in the Santa Barbara region to the south (Site 893, 34.1°N) matches with the 17- to 14.5-ka Lake Lahontan-Bonneville highstand.

Two wet periods should be found in central California if westerly storm tracks were displaced: one before the LGM as the Northern Hemisphere glaciers advanced and the storm tracks moved to the south, and a second one as the storm tracks moved northward again. However, at Site 1018 arid conditions persisted for the entire period between ~35 and 12.5 ka (8). Only in southern California (Site 893) is there evidence for moderately higher precipitation before the LGM, between 45 and 25 ka (24).

In the western Pacific, the westerly belt did not shift dramatically southward, implying little or no shift in the east near North America and little southward compression of the North Pacific subtropical gyre. Rea and Leinen (31) found that the amount of Asian dust carried to the oceans changed by a factor >1.5 over the past 30 ky, but the position of maximum dust flux marking the westerlies remained between 38 and 40°N. Yamamoto *et al.* (32) delineated the northern

margin of the Kuroshio (the western Pacific boundary current) for 0 to 25 ka by SST and estimated that the maximum glacial southward compression of the gyre to be $\sim 1.4^\circ$.

California coastal SST patterns (Fig. 1) do not fit with south-displaced winter storm tracks at the LGM. An enlarged subarctic gyre in association with a southward displacement of the subtropical gyre should cool SST to the south along the coast of Baja California. However, between 25 and 12.5 ka, a steep SST gradient was present between San Francisco and Santa Barbara [temperature change (ΔT) $\approx 5^\circ$] (18), compared with 0.9° during the Holocene (Fig. 1). Southern California SST warmed earlier than SST to the north (~ 25 ka), which is indicative of weak flow in the California Current (18). The warm Pleistocene SST pattern off southern California and large SST gradient off central California implies southerly winds to southern California, not westerlies.

Last, the LGM vegetation assemblage in southern California was not one that should have appeared with increased westerly storms. In the Holocene, coastal redwood (*Sequoia*) can only be found to the north of 36° N, where temperatures are cooler than in southern California and water is more available. At the LGM, coastal cooling was sufficient for redwoods to expand southward, but they did not because Southern California was still arid, just not as arid as in the Holocene (21).

“Out of the tropics.” The different timing of wet intervals on either side of the Sierra Nevada Mountains implies major water transport into the Great Basin from the south during the deglaciation (Fig. 4). North of 36° N, coastal climates were cooler and drier when Lake Lahontan and Bonneville reached their highest levels. The Lake Lahontan/Bonneville highstands between 17 and 14.5 ka were unsupported by additional precipitation travelling east across the Sierra Nevada. Even in high-resolution climate model experiments that show a “split” jet-stream and stronger Pacific cyclogenesis in response to LGM boundary conditions and CLIMAP-based SSTs, the enhancement of westerly storm tracks is confined to the subarctic North Pacific (12).

The temporal and spatial distribution of lake highstands fits with warm wet air masses moving north into the Great Basin from the eastern tropical Pacific. In the Holocene, coastal southern California and northern Baja California have persistent upwelling associated with northerly winds around the northeast Pacific subtropical high-pressure regime that cools SST and blocks tropical air masses (33). The warm ocean conditions found south of 34° N from ~ 25 ka until ~ 10 ka (Fig. 1) (18) are an indication that the conditions that drove upwelling were suppressed and imply that the northeast Pacific high was also less effective at blocking southern storms. Evidence for this scenario can be found by comparing the Devils Hole ^{18}O time series to coastal SST records. Maximum tropical source water, as

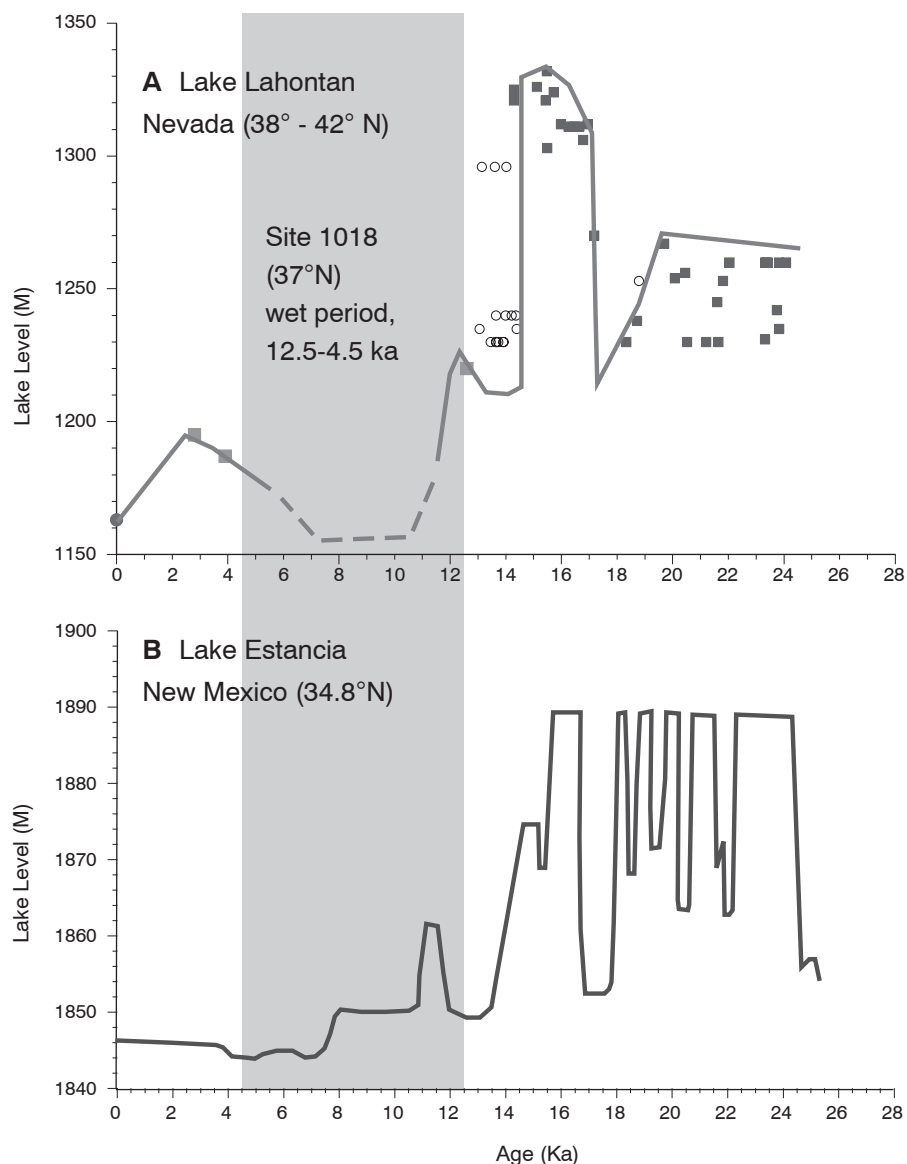


Fig. 3. Lake level records in thousands of calendar years before present from (A) Lake Lahontan, Nevada (5, 41, 42) and (B) Lake Estancia, New Mexico (43) compared with the Site 1018 wet period (gray shading). Site 1018, being west-southwest of Lake Lahontan, should respond to any substantial precipitation that came directly across the Sierra Nevada mountains to the lake. The two lake level records show that the early wet interval was found in both the central Great Basin and New Mexico, and that the 17- to 14-ka wet interval was magnified in the central Great Basin.

indicated by “warm” $\delta^{18}\text{O}$, is found at 17 ka (34), when all SST paleotemperatures in the California Borderlands are as warm or warmer than modern values (Fig. 1).

Seasonality of deglacial wet intervals. During the Holocene, southern-sourced precipitation annually appears with the summer North American monsoon, which is strongest in northwest Mexico, New Mexico, and Arizona (35). The deglacial tropical water source may well result from an enhancement of this summer precipitation pattern. Coastal precipitation and SST patterns imply that the northeast Pacific high may have been displaced or weakened relative to modern conditions, allowing wet Pacific trop-

ical air more access into the Great Basin at its southern end. A weaker northeast Pacific high should have helped to enhance the summer precipitation by weakening winds that might block access of tropical Pacific air masses to the Great Basin. Enhanced contribution of moisture from the Gulf of Mexico associated with the summer monsoon could also increase moisture in the Great Basin without triggering a wet interval along the Pacific coast, and is therefore also consistent with the observed proxy data.

Wet conditions peaked earlier near Mexico relative to the rest of the Great Basin. Somewhat higher lake levels before 17 ka were found throughout much of the Great Basin, but highest

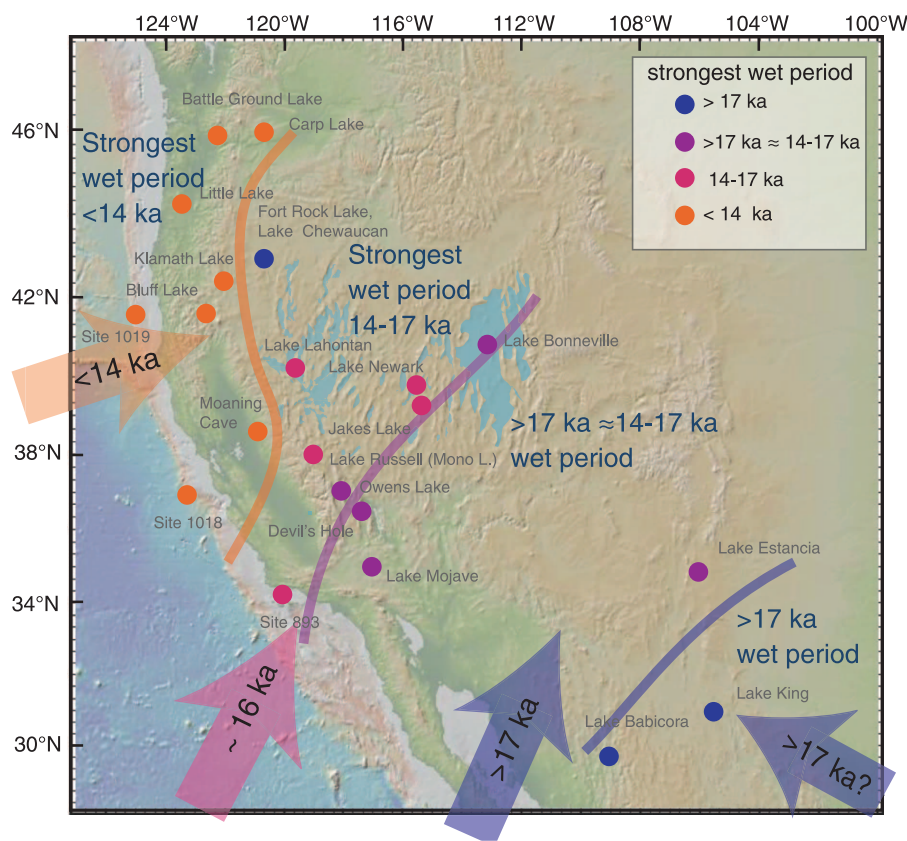


Fig. 4. Timing of lake level changes and coastal wet periods in western North America (table S1). Multiple wet periods are found over the LGM and deglacial intervals. The largest events typically occur earliest in the southwest and later to the north. For a given latitude, the coastal region has wet periods persistently later than the interior western United States. Arrows represent possible storm tracks.

lake levels in the south typically occurred before the 17- to 14-ka period of highest lake levels in the central Great Basin (see supplementary text). In the 17- to 14-ka time interval, coastal California did not receive higher levels of precipitation. Better penetration of tropical air into the central Great Basin through southern California and the Gulf of California, perhaps drawn by a stronger summer monsoon, would create a precipitation pattern such as the one observed. The time of maximum lake level in Lake Lahontan occurs when there is evidence of strong easterly winds in Oregon (36, 37) peaking at ~15 ka. The easterlies are evidence of the regular development of a low-pressure regime to the south of Oregon, perhaps associated with the summer monsoon. In support of the summer monsoon hypothesis, McClymont *et al.* (38) proposed that 15 to 17 ka represented a period of higher precipitation around the Gulf of California and used modeling to suggest that there was a strengthened summer monsoon at this time.

With the data now in existence, it is impossible to determine whether summer precipitation was more enhanced than winter precipitation between 17 and 14 ka. However, if winter storms were the major precipitation source, it is difficult to understand why coastal California remained dry.

Conclusions. Climate change associated with the deglaciation caused profound alterations of

the western U.S. water cycle. Large lakes formed in the central Great Basin, mainly caused by increased precipitation. The timing of highest lake levels in the Great Basin are not synchronous, but have a progression from south to north that does not coincide with the northward progression of wet intervals along the U.S. west coast. Instead, highest lake levels for Lakes Lahontan and Bonneville match the timing of a wet interval found in southern California, but not with wet intervals on similar latitude bands. The evidence suggests that precipitation in the glacial western United States originated from the tropical eastern Pacific, perhaps via stronger spring/summer precipitation fed by tropical air masses rather than higher numbers of westerly winter storms.

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Supplementary Materials

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Supplementary Text

Table S1

References (44–80)

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Parallel Molecular Evolution in an Herbivore Community

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Numerous insects have independently evolved the ability to feed on plants that produce toxic secondary compounds called cardenolides and can sequester these compounds for use in their defense. We surveyed the protein target for cardenolides, the alpha subunit of the sodium pump, Na⁺,K⁺-ATPase (ATPα), in 14 species that feed on cardenolide-producing plants and 15 outgroups spanning three insect orders. Despite the large number of potential targets for modulating cardenolide sensitivity, amino acid substitutions associated with host-plant specialization are highly clustered, with many parallel substitutions. Additionally, we document four independent duplications of ATPα with convergent tissue-specific expression patterns. We find that unique substitutions are disproportionately associated with recent duplications relative to parallel substitutions. Together, these findings support the hypothesis that adaptation tends to take evolutionary paths that minimize negative pleiotropy.

Evolutionary genetics has long aimed to understand both the limits of the rate of evolution and the extent to which evolutionary paths are predictable (1). Although it is often presumed that adaptive evolution is mutation-limited (2), others have emphasized the role of pleiotropic and epistatic effects in constraining evolution. In particular, it has been proposed that (i) adaptation may tend to involve regulatory, rather than protein, changes because the former may be less pleiotropic (3); (ii) for similar reasons, specific genes may be hotspots for adaptation (4); (iii) because of epistasis, major evolutionary transitions may be facilitated by small population sizes (4–6); and (iv) that gene duplication may play an important role in the evolution of novelties by releasing constraints imposed by pleiotropy (6–8). The relative importance of each of these factors in organismal evolution is still debated (1, 3, 4, 8, 9). Although there are specific examples for each of these effects, they are often difficult to compare directly because they occur in very different contexts (10–13). Evolutionary parallelisms in settings where different species repeatedly face a common selective pressure provide a natural experiment for addressing these questions, which allows one to assess the extent to which phenotypic changes are caused by the recruitment of a limited set of genes and to test whether the specific molecular changes observed involve identical (or highly similar) substitutions (9).

One example of convergence involves the ability of the larvae and adults of a variety of insect species to feed on cardenolide-producing plants of the family Apocynaceae, notably in the genera *Asclepias* (“milkweed”) and *Apocynum* (“dogbane”) (14). Among other antiherbivore adaptations

(15), these plants produce a class of secondary metabolites called cardenolides that are toxic to most herbivores. Cardenolides bind to and inhibit the alpha subunit of the sodium pump, Na⁺- and K⁺-exchanging ATPase (Na⁺,K⁺-ATPase) (ATPα), part of a multisubunit active transporter of Na⁺ and K⁺ that is crucial for muscle contraction, neural function, and other animal cellular processes requiring cation transport. In humans and other mammals, naturally occurring cardenolides regulate the activity of Na⁺,K⁺-ATPase in the context of many physiological processes, and plant-derived cardenolides have been used medicinally for hundreds of years (16). Within insects, herbivores feeding on Apocynaceae include several taxonomic orders spanning 300 million years of insect evolution. Beyond obtaining nutrition from these plants, some of these insects sequester cardenolides for their own defense against predation and have evolved associated aposematic coloration to advertise their unpalatability (17). Perhaps the best-known example is monarch butterflies (*Danaus plexippus*), whose aposematic larvae feed on milkweed plants and sequester large amounts of cardenolide, which in turn provides protection to the larvae and adults from a variety of predators (18).

Insect-host specialization on Apocynaceae plants includes both behavioral and physiological adaptations (15, 19), including amino acid changes in ATPα. Monarch butterflies carry at least one amino acid substitution (N122H) in ATPα that confers resistance to the inhibitory effects of cardenolides (20, 21). Additionally, the same substitution (22), as well as a second substitution (L111V) (23), is shared between Danaid butterflies and Chrysomelid beetles, which suggests parallel molecular evolution in these taxa. However, resistant forms of the enzyme in other taxa (e.g., *Rattus norvegicus*, *Hydra vulgaris*, and *Bufo* spp.) lack these specific substitutions; this implies that parallel evolution of cardenolide insensitivity is not always due to the same amino acid substitutions (24–26).

A limitation in examining cardenolide-resistant forms of ATPα, with the exception of Danaid butterflies and Chrysomelid beetles, is that resistance did not arise in the same, or even a similar, ecological context. Additionally, previous work has focused on the 12-amino acid H1-H2 extracellular domain of ATPα (20–23, 26, 27), yet mutagenesis studies have revealed at least 23 residues scattered throughout the ~1000-amino acid ATPα protein at which amino acid substitutions confer some degree of reduced sensitivity to cardenolides (fig. S1). Structural studies have identified 17 residues that likely directly interact with the cardenolide ouabain or stabilize its binding (28–31), including 12 not detected in mutagenesis studies. Moreover, the states for most residues outside of the H1-H2 extracellular domain have not previously been investigated in cardenolide-sequestering insects (with the exception of the monarch butterfly) (32).

Recurrent gene duplication in Apocynaceae-associated taxa. We investigated the evolution of the ATPα protein among diverse taxa feeding on Apocynaceae plants, which are subject to similar selective pressures driving the evolution of insensitivity to cardenolides. The genomes of the fruit fly (*Drosophila melanogaster*), pea aphid (*Acyrtosiphon pisum*), monarch butterfly, and flour beetle (*Tribolium castaneum*) contain two, two, three, and four copies of ATPα, respectively, representing two ancient, distinct lineages (which we refer to as ATPα1 and ATPα2). This duplication likely dates back to the diversification of arthropods (fig. S2). However, the current assembly of the silk moth (*Bombyx mori*) genome contains just one detectable copy of ATPα that falls in the ATPα1 clade, which suggests that either the silk moth genome is incomplete or that these ancient duplicates have not persisted in all taxa. In monarch and fruit fly, ATPα2 genes have relatively low levels of expression (32) (<http://flybase.org/reports/FBgn0040037.html>).

We focused on the evolution of ATPα1, the copy common to all of the insects surveyed here, which is evolutionarily constrained at sites implicated in ouabain-binding in outgroup taxa. We performed a survey based on mRNA-Seq transcriptome shotgun sequencing of 26 taxa [including 14 that feed on Apocynaceae (table S1) (33)], as well as an analysis of the complete genomes of pea aphid, silk moth, monarch butterfly, and red flour beetle (33), and we detected single copies of ATPα1 in all but four taxa. Two copies of ATPα1 (designated ATPα1A and ATPα1B) [see (Fig. 1)] were identified in dogbane beetles (*Chrysomelid auratus*) and milkweed stem weevils (*Rhyssomatus lineaticollis*), and we detected three copies (designated ATPα1A to C) (Fig. 1) in large and small milkweed bugs (*Oncopeltus fasciatus* and *Lygaeus kalmii*, respectively). Our results imply that at least four recent duplications of ATPα1 have occurred in lineages that feed on Apocynaceae hosts, and none have occurred in lineages that do not [the duplication in the dogbane beetle was overlooked in a previous study (22)]. We estimate that the most ancient of

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these duplication events, found in large and small milkweed bugs, occurred ~60 million years ago (Mya) for copies A and B and ~125 Mya for copies C and the ancestor of A and B (33). The estimated divergence time for the gene copies A to C exceeds the divergence between the large and small milkweed bugs (~30 Mya), which implies that all three duplications are likely shared by many species within the Hemipteran subfamily Lygaeidae. The duplications of ATP α 1 observed in the dogbane beetle and the milkweed stem weevil are considerably younger (~24 and ~9 Mya, respectively) but may also include multiple species (33).

Accelerated protein evolution in Apocynaceae-associated taxa. A signature of adaptive protein evolution is apparent in patterns of substitution at sites of ATP α 1 implicated in ouabain binding (Fig. 1). Specifically, 33 of the 49 inferred amino acid substitutions occur in lineages that use Apocynaceae (including feeding ability and sequestration of cardenolides) ($P = 1.7 \times 10^{-7}$, accounting for branch lengths sampled) (33). Note that aphids show a distinct pattern (Fisher's exact test, $P = 1.5 \times 10^{-7}$), with all 11 ATP α 1 substitutions having accumulated before the diversification of the oleander aphid and the pea aphid (~65 Mya) (33). If we exclude the aphid-whitefly lineage, the association between amino acid substitutions and use of Apocynaceae therefore becomes stronger, with 33 of 34 substitutions occurring on lineages

that use Apocynaceae (Lepidoptera: 5 of 6 substitutions; Coleoptera: 16 of 16 substitutions; Hemiptera: 12 of 12 substitutions; $P = 7.9 \times 10^{-10}$, accounting for branch lengths sampled) (33).

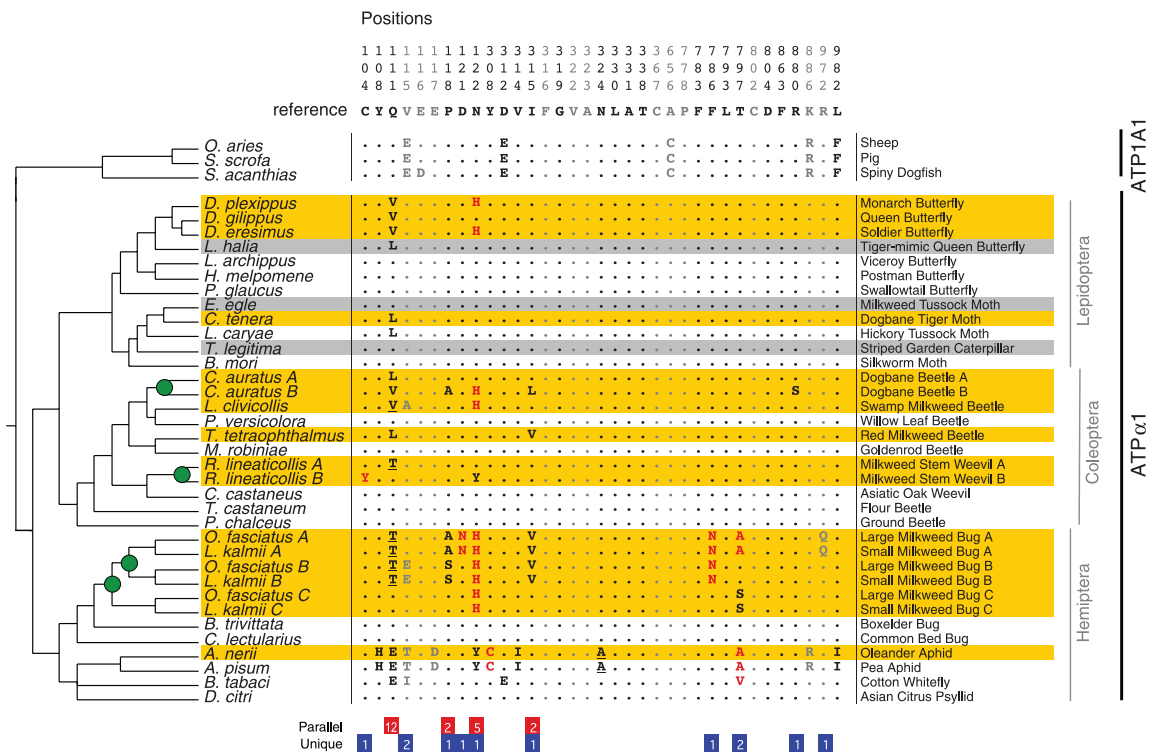
We also see considerable parallelism at the level of amino acid substitution in species that use Apocynaceae. In particular, glutamine at position 111 has experienced 12 substitutions, all of which are observed in at least one other lineage (Fig. 1). Also, position 122 has a replacement of asparagine by histidine in five independent lineages. These observations suggest that sites 111 and 122 are hotspots for substitution in Apocynaceae-feeding lineages (binomial test $P = 6.0 \times 10^{-11}$ and $P = 3.1 \times 10^{-4}$, respectively) (33). Overall, substitutions are significantly clustered in the H1-H2 extracellular domain (i.e., 24 of 33 substitutions occur at positions 111 through 122, binomial test, $P = 9.5 \times 10^{-11}$).

Unique substitutions are associated with duplications. Under a model in which pleiotropic effects constrain evolution, gene duplications may facilitate substitutions that would be deleterious in organisms with a single copy (7, 8). Consistent with this hypothesis, of the substitutions that have occurred on lineages associated with the use of Apocynaceae, unique substitutions are almost exclusively observed in lineages that carry a recent duplication of ATP α 1 (11:1, $P = 0.017$, accounting for branch lengths sampled) (33), whereas parallel substitutions show no such pattern

(11:10, $P = 0.67$, accounting for branch lengths sampled) (33). Comparing the ratios above confirms that parallel and unique substitutions show significantly different patterns of substitution with respect to duplication status of ATP α 1 (Fisher's exact test, $P = 0.027$).

The molecular basis for insensitivity to cardenolides. In a handful of cases (e.g., C104Y, D121N, N122H, Y308C, F786N, and T797A in Fig. 1), we know the effects of particular substitutions on cardenolide-binding affinity from site-directed mutagenesis studies (fig. S1). However, for most substitutions associated with the use of Apocynaceae, we lack this type of detailed information. To examine the likely effects of substitutions in ATP α 1 across insects, we used molecular docking simulations with the crystal structure of the pig ortholog of ATP α bound to the cardenolide ouabain (Fig. 2A) (33). We compared ouabain docking onto the wild-type pig protein with docking onto a protein modified to incorporate individual amino acid substitutions that occurred in lineages associated with use of Apocynaceae (Fig. 1). We show that N122H, which has arisen separately in five lineages and is known from mutagenesis studies to confer resistance to ouabain in cell lines (21), likely acts by sterically blocking ouabain from entering the steroid-binding pocket of ATP α (Fig. 2B). Our docking simulations suggest that four other substitutions (C104Y, N122Y, R880S, and R972Q)

Fig. 1. The phylogenetic pattern of amino acid substitution at sites implicated in cardenolide-binding for ATP α 1 in taxa that feed on Apocynaceae and outgroups. Numbered columns correspond to sites for which site-directed mutagenesis or protein structure analysis has suggested a role in cardenolide-binding (fig. S1). Gray sites correspond to those with only structural evidence for a role in cardenolide-binding. Dots represent identity with the consensus sequence. Letters in red are specific substitutions characterized in functional assays (fig. S1). Underlined are substitutions that require at least two nonsynonymous substitutions relative to the ancestral sequence. Orange-shaded rows correspond to specialists known to sequester cardenolides. Gray-shaded rows represent species that either are not known to sequester cardenolides and/or are nonspecialists only occasionally found on Apocynaceae. The cladogram represents the accepted species branching order (table S6), and branch lengths are not meaningful. Green circles represent in-



have similar effects on ouabain binding (see Figs. 1 and 2C, fig. S3, and table S2). Of these, C104Y is known from functional studies to reduce the affinity of ATP α for ouabain to one-sixth of normal (fig. S1). Thus, although these amino acid substitutions differ, they appear to be functionally parallel. Most other observed substitutions are predicted to have more subtle effects (table S2). For example, the substitution Q111V, which appears in three independent lineages, appears to allow ouabain to fully penetrate the binding pocket (Fig. 2D), although it likely disrupts a key hydrogen bond between the protein and the steroid core of ouabain (Fig. 2A).

Because the ATP α of vertebrates shares only ~77% amino acid identity with insects, it is possible that, in insect lineages, substitutions that ameliorate the ouabain-inhibitory effects of some of the substitutions have arisen elsewhere in the protein. To address this possibility, we performed ouabain-docking simulations on predicted structures for the 19 native proteins of the taxa associated with Apocynaceae (fig. S3) (33). We found a significant positive correlation between the number of substitutions at sites implicated in ouabain-binding (Fig. 1) and the extent of displacement of the best ouabain docking (Spearman rank correlation test, $P = 0.007$) (table S3). Further, the 11 native proteins with one or more substitutions predicted to have a large effect (table S2) exhibit significantly greater displacement of ouabain than the eight native proteins lacking such substitutions (Mann-Whitney U test, $P = 8 \times 10^{-4}$) (table S3). Finally, we show that the best dockings for ouabain to predicted native protein structures with one or more large effect substitutions are further displaced relative to revertant forms of the native proteins in all 11 cases (Sign test, $P = 0.001$) (table S4).

Differential expression of ATP α 1 duplicates.

The docking simulations that we performed allow us to predict the extent of ouabain sensitivity of duplicate copies of ATP α 1. Of these duplicates, we expect higher expression of the putatively less sensitive copy in the gut, as this is the primary location of cardenolide processing in these species. In contrast, the copy with the highest level of sensitivity to cardenolides should occur in the brain, because a glial sheath surrounding nervous tissue likely functions as a barrier that prevents cardenolides from reaching Na⁺,K⁺-ATPase in the ventral nerve cord (34). In support of this hypothesis, in the Asian grasshopper *Poecilotherus bufonius*, which has tissue-specific variation in cardenolide sensitivity, Na⁺,K⁺-ATPase in the brain was found to be the most sensitive to ouabain inhibition compared with Na⁺,K⁺-ATPase of the gut and excretory system (35).

In the dogbane beetle, ATP α 1B has accumulated five substitutions at sites in the protein that are predicted to determine ouabain-binding affinity (Fig. 1), including N122H and R880S. Docking simulations of ouabain on the predicted structure of native ATP α 1B suggest significant displacement of ouabain relative to ATP α 1A (Fig. 3A

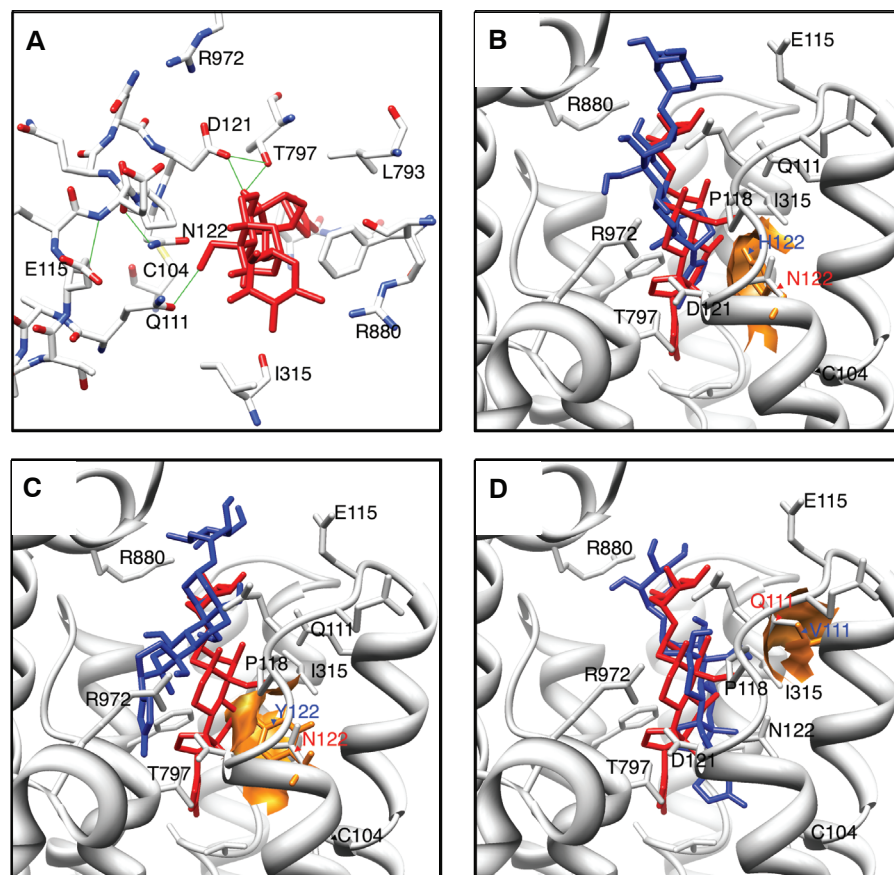


Fig. 2. Cardenolide-binding pocket structure and molecular docking-simulation results. **(A)** Structure of the cardenolide-binding pocket of ATP1A1 for *Sus scrofa* bound to the cardenolide ouabain (in red) from (31). Green lines represent possible hydrogen bonds between the protein and ouabain. **(B to D)** Docking simulation results for the substitutions **(B)** N122H, **(C)** N122Y, and **(D)** Q111V. In each case, we show the best docking position for ouabain docked to the native protein (in red) versus the protein with one of three simulated substitutions (in blue). The best docking position is defined as that closest to the coordinates of ouabain in the published cocrystal structure (31). The atomic surfaces of the derived amino acid side chains at position 111 and 122 are detailed in gold. N122H and N122Y sterically block ouabain from entering the binding pocket [root mean square deviations (RMSDs) from cocrystal position are 5.5 and 6.9 Å, respectively; RMSDs from the best wild-type docking position are 5.6 and 6.9 Å, respectively]. Three other substitutions (C104Y, R880S, and R972Q) are predicted to have similar effects (table S2). In contrast, Q111V has more subtle effects on the position of ouabain (RMSD is 2.98 Å from the cocrystal structure and 0.14 Å from the best wild-type docking position) but likely disrupts a stabilizing H-bond (A).

and table S3) and imply that ATP α 1B is likely to be less sensitive to ouabain inhibition than ATP α 1A. These two copies are differentially expressed among tissues (Fig. 3A). The relatively cardenolide-insensitive ATP α 1B of the dogbane beetle is expressed seven times as much as ATP α 1A in the digestive tract (Fig. 3A). Conversely, the sensitive ATP α 1A has three to six times the expression of the less-sensitive ATP α 1B in the head and thoracic muscle.

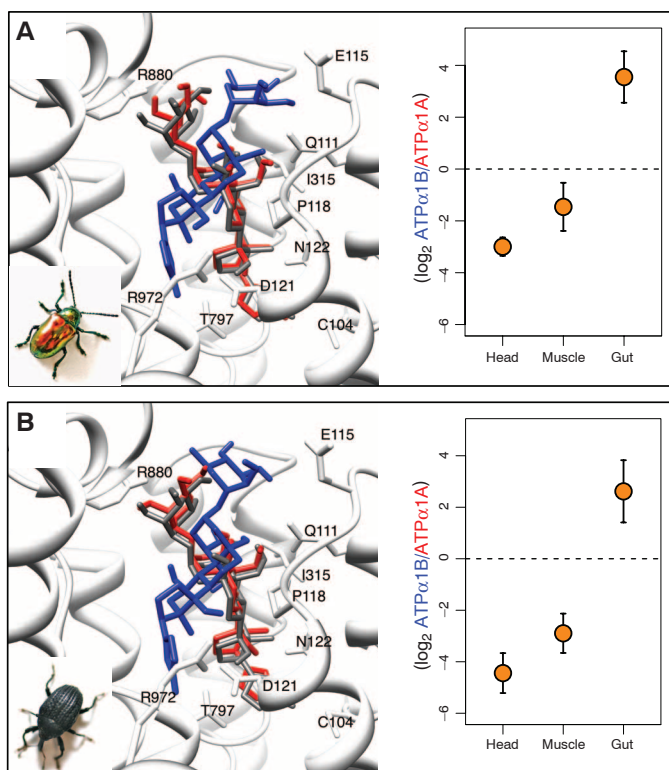
Similarly, ATP α 1B of the milkweed stem weevil harbors two substitutions (C104Y and N122Y) that are predicted to diminish its sensitivity to cardenolides. Our docking simulations imply that this copy of ATP α 1 is also likely to be less sensitive to ouabain inhibition than ATP α 1A, and the two copies have a pattern of differential expression that is almost identical to that of dogbane beetles (Fig. 3B). We also found the same

rank-order of tissue-specific expression of putatively less sensitive to more-sensitive copies of ATP α 1 in large and small milkweed bug, where the sensitivity of the three duplicates of ATP α 1 can be ranked by the total number of substitutions at sites implicated in ouabain-binding and the number of substitutions with large effects identified in docking simulations (table S5 and fig. S4).

Thus, in addition to parallelisms in gene copy number and at the level of individual amino acid substitutions, we also observe parallel evolution of expression patterns for ATP α 1 duplicates arising in distantly related taxa. Differential expression of ATP α 1 duplicates in these taxa may have facilitated the functional specialization of what is otherwise a highly conserved protein.

Discussion. Our results suggest that adaptive evolutionary paths are, at least to some extent, predictable. ATP α 1 is clearly a hotspot for molecular

Fig. 3. Native protein–docking simulations and tissue-specific gene expression for ATP α 1 duplicates in (A) dogbane beetles and (B) milkweed stem weevils. (Left) Best docking results for ouabain onto pig ATP1A1 (gray ligand) and estimated structures for ATP α 1A (red ligand) and ATP α 1B (blue ligand) for each species (33). For both species, the best dockings of ouabain to the predicted structure of native ATP α 1A are within 1 Å RMSD of the best docking to pig ATP1A1 (table S3). In contrast, the best dockings of ouabain to putatively resistant ATP α 1B copies for dogbane beetles (A) and milkweed stem weevils (B) are displaced by 5.9 and 6.2 Å RMSD, respectively. (Right) Tissue-specific expression means (with two standard errors) of estimates for three unrelated individuals. Positive values indicate higher expression of the putatively resistant copy, ATP α 1B. Analysis of variance (ANOVA) reveals significant variation in expression pattern among tissues (dogbane beetles: $F = 74.3$, $P = 6 \times 10^{-5}$; milkweed stem weevils: $F = 21.1$, $P = 4 \times 10^{-4}$). A Tukey–Kramer test reveals significant differences in all pairwise comparisons except head versus muscle (dogbane beetles, gut–head: $P = 6 \times 10^{-5}$; gut–muscle: $P = 3 \times 10^{-4}$; head–muscle: $P = 0.08$; weevils, gut–head: $P = 4 \times 10^{-4}$; gut–muscle: $P = 3 \times 10^{-3}$; head–muscle: $P = 0.36$).



evolution associated with insect adaptation to using Apocynaceae, as seen by repeated duplication of ATP α 1, parallel changes in gene expression, and parallel amino acid substitutions. The basis for this parallel evolution seems most readily explained by negative pleiotropic effects of most amino acid substitutions in this protein. In particular, we observed that unique substitutions are mostly confined to lineages with duplicate copies of ATP α 1 and that 9 of 11 amino acid substitutions in lineages with one copy occur at only two sites (111 and 122). This level of parallelism is consistent with the hypothesis that negative pleiotropic effects prevent changes at other amino acid sites that could theoretically contribute to reduced sensitivity of ATP α 1 to cardenolides.

Despite the large number of parallelisms observed in the evolution of this system, the pattern in aphids (Fig. 1) indicates that not all substitutions conferring reduced sensitivity to cardenolides at ATP α 1 are necessarily related to adaptation to cardenolide-containing host plants. Notably, the ATP α 2 of aphids is ancestral at many sites implicated in cardenolide binding, contrary to all other insects surveyed here (fig. S5). In addition, ATP α 2 of aphids lacks introns, whereas the fruit fly, flour beetles, and monarchs retain them, which suggests that the ATP α 2 of aphids may represent a retrotransposed copy of the original duplicate.

On this basis, we speculate that ATP α 1 and ATP α 2 may perform different roles in aphids than in other insects.

A decade-old debate centers around the relative importance of protein versus regulatory changes and the role of gene duplication in the evolution of novelties [e.g., (3, 8)]. Although our findings point to a certain level of predictability in evolution, they also reveal a plurality of evolutionary responses to a similar selective pressure, which demonstrates how adaptive evolution can leverage a combination of gene duplication, regulatory changes, and protein evolution. The genetic basis of repeated adaptations can be further elucidated by considering contexts where diverse groups of species are faced with a common selective pressure, such as insect–host specialization; cryptic melanism; or environmental gradients in oxygen, salinity, temperature, or acidity.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6102/1634/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S8
Tables S1 to S11
References (36–80)

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The Precise Solar Shape and Its Variability

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The precise shape of the Sun has not been convincingly determined, despite half a century of modern photoelectric observations. The expected deviation of the solar-limb shape from a perfect circle is very small, but such asphericity is sensitive to the Sun's otherwise invisible interior conditions, as well as the solar atmosphere. We use evidence from a long-running experiment based in space to show that, when analyzed with sufficiently high spatial resolution, the Sun's oblate shape is distinctly constant and almost completely unaffected by the solar-cycle variability seen on its surface. The solar oblateness is significantly lower than theoretical expectations by an amount that could be explained by a slower differential rotation in the outer few percent of the Sun.

The magnitude of the solar oblateness (expressible as a pole-equator radius difference or a Legendre polynomial shape coefficient) is a fundamental quantity and is potentially a constraint on theories of gravity (1). The solar-limb shape responds to the solar gravitational potential, the Sun's interior rotation, and magnetic and fluid-flow stresses at the visible photosphere. Modern observations over the past half-century have concentrated on determining the Sun's shape, or solar oblateness, but various instruments on the ground and space have not yielded consistent measurements (Fig. 1) (1–8). The discrepant measurements have been interpreted as evidence that the Sun's shape varies with the solar cycle (3, 7, 9) or that it may be dominated by surface magnetic contamination (8).

The Helioseismic and Magnetic Imager (HMI) aboard NASA's Solar Dynamics Observer (SDO) is a satellite instrument unique in its capability to observe the solar limb. The HMI benefits from the experience of NASA's Solar and Heliospheric Observatory and Michelson Doppler Imager (SOHO/MDI) experiment, which provided previous sensitive, space-based solar-limb-shape measurements (10). HMI has 16 times as many detector pixels as MDI and uses precise thermal control of the instrument and its optics. Because the SDO spacecraft and HMI undergo semiannual roll calibrations, the HMI can yield very sensitive measurements of the solar-limb-shape variability. From its geosynchronous orbit, the HMI generates more than 15,000 solar images per day and is immune from the optical distortion effects of the terrestrial atmosphere. Solar measurements from the past two years are particularly important for understanding how magnetism may affect the Sun's

shape, either through global solar-cycle changes or by superficial effects in the magnetized outer solar atmosphere. During this time, the Sun has evolved from its extended magnetic minimum, when it exhibited periods with no observable sunspots or solar activity, to its present approach toward maximum, where daily sunspot numbers have exceeded 90.

Observations of the solar disk from the ground suffer from terrestrial atmospheric blurring and distortion noise at the arc second scale. Over the 0.5° angular diameter of the Sun's disk, this noise is incoherent around the limb. In contrast, from space the dominant limb-shape noise comes from spacecraft pointing and optical distortion. Because these errors are either coherent across the image or temporally slowly varying, they can be removed to yield a precise solar shape that may be orders of magnitude more accurate than that obtained from ground-based data. For the SOHO/MDI satellite, these corrections were made by combining multiple images while the satellite rotated with respect to the Sun-pointing direction (7, 10). This

allowed the solar distortion, which is ~10 milli-arc sec, to be separated from nonaxisymmetric optical distortions, which could be more than 20 times larger. Previous MDI measurements showed that the limb position and brightness may be accurately extracted from image pixels by evaluating the radial and angular dependence of the limb-darkening function (LDF), which we empirically determine from each image. The derived image-mean LDF, $\Gamma(r)$, is the template and reference for measuring local limb position and brightness variations. It is critical for obtaining the solar-limb distortion function. We determined this function with the use of least-squares techniques (11), which took as input the HMI 4-by-4-K pixel images with an image scale of 0.5 arc sec per pixel (12). The brightness and shape were then obtained in 180 (2° wide) θ bins.

Previous MDI measurements (7, 10) were obtained from relatively defocused, 2-arc sec per pixel sampled data and suffered from blurring caused by instrumental distortion. The instrument point-spread function (and effective angular resolution) was also temporally more variable than HMI because of limited temperature control. The results presented here are more accurate because of HMI's improved stability and because our analysis corrects for distortion before evaluating the template LDF, $\Gamma(r)$ (Fig. 2). The spatial resolution increased between the MDI and HMI experiments from ~2.5 to better than 1 arc sec.

True solar and instrument contributions to the apparent limb shape were distinguished by rolling the SDO spacecraft in 16 or 32 steps of 22.5° or 11.25°, respectively. The solar signal rotated through the data as SDO rolled while the instrument noise was fixed to the satellite reference frame. Full disk images were obtained at several narrow (7.6 nm) wavelength bands and in various Stokes polarization states at each SDO roll angle. This analysis used the far-red HMI continuum pass-band and the circularly polarized limb data, but the results were insensitive to the optical

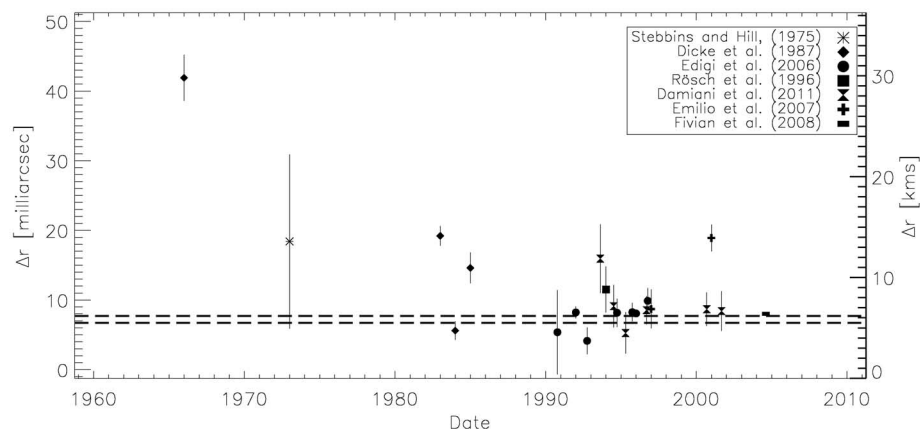


Fig. 1. Summary of modern photoelectric solar oblateness measurements. The difference in apparent equatorial and polar radii (Δr) versus the mean observation date is plotted. The dashed lines show the 1 σ limits of the precise value obtained from the HMI series of measurements.

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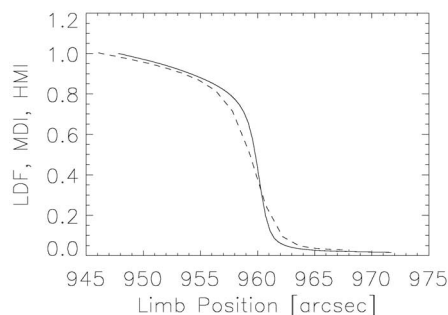


Fig. 2. Solar limb spatial resolution improvement with HMI. The solid curve shows the normalized HMI LDF, $\Gamma(r)$, whereas the dashed curve shows the MDI LDF. The steeper slope of the HMI function is due to the considerably higher spatial resolution of HMI, which clearly resolves the steep edge in the LDF.

polarization. Six SDO/HMI 7-hour roll sequences have been obtained. HMI provided nearly simultaneous measurements from two separate charge-coupled device cameras that observed different optical polarization states (12), but notably, the two resulting local limb position and brightness data sets were identical to within $\sim 3 \times 10^{-4}$ of a pixel and 2×10^{-5} of the mean limb brightness (13). The dominant HMI limb noise came from the intrinsic solar-limb roughness and brightness variability with uncorrected instrument noise that was one to two orders of magnitude smaller.

Our high-resolution analysis effectively separates (and decorrelates) local LDF brightness from the measured limb displacement in all bins that are not “bright.” The low-resolution MDI analysis (7, 10) shows that brighter limb regions move the limb inward at all signal levels. With HMI, only outlier data bins brighter than $\sim 0.5\%$ affect the limb position. This excess brightness is associated with magnetic regions and a real change in the solar atmosphere known as the Wilson depression (14). In HMI data, the Wilson depression appears as an inward shift of the solar limb of ~ 0.02 pixels or 10 milli-arc sec in bins with a brightness excess of 0.5% . With the greater sensitivity of HMI, smaller limb brightness values show no correlation between brightness and limb position, unlike results from MDI (15). Simply removing these bright data bins from the global shape analysis eliminates magnetic contamination of the limb shape to the level of our noise threshold of less than 0.5 milli-arc sec in the oblateness. Depending on the solar activity, including these bins can bias the oblateness by as much as a few milli-arc seconds. Less than 5% of the HMI data were deleted in this way. This finding contrasts with the Reuven Ramaty High-Energy Solar Spectroscopic Imager oblateness procedure performed by Fivian *et al.* (8), which removed $\sim 80\%$ of their measurements from analysis.

We divided the brightness-corrected limb data into 12 angular bins from which we evaluated the mean and standard deviation of the limb position. We then obtained the results for six spacecraft

Fig. 3. Binned continuum wavelength, circular polarization, and limb-shape data from roll 1 (table S1). The dashed curve shows the oblateness and hexadecapole fit to the HMI shape versus angle. Error bars indicate statistical 1σ errors from these roll data.

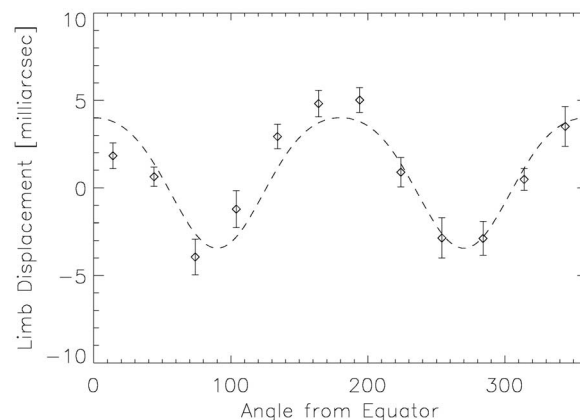
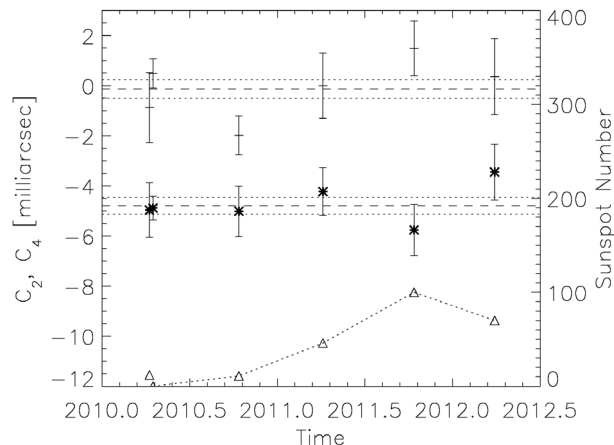


Fig. 4. Oblateness (asterisks) and hexadecapole (dashes) coefficients versus time. The dashed and dotted lines show the mean and standard error, respectively, of the weighted average of these shape amplitudes. Triangles denote the 3-day mean sunspot numbers (right y axis) centered at the time of the HMI measurements. Error bars indicate 1σ statistical errors derived from the measurements.



rolls, collected over a 2-year period, from a weighted fit to a quadrupole (oblateness, C_2) and fourth-order Legendre polynomial (a hexadecapole, C_4) (Fig. 3). The final temporal variations of the oblateness and hexadecapole solar-shape terms are shown in Fig. 4, along with the corresponding sunspot numbers. The implied difference between the equatorial and polar solar radii due to these shape terms is $-3/2$ of the oblateness and $-5/8$ of the hexadecapole amplitude. Predicted dimensionless shape coefficients (C_2 and C_4 were normalized by the solar radius) have been calculated from the solar interior-rotation profile (16) and can be compared with these data by dividing the Legendre polynomial amplitudes by a nominal solar radius of 960 arc sec.

The HMI oblateness coefficient is -4.80 ± 0.33 milli-arc sec (corresponding to an equator-pole radius difference of 7.20 ± 0.49 milli-arc sec) and rules out the possibility of a substantial solar-cycle oblateness variation of more than 0.1 milli-arc sec during the rising phase (17). The oblateness is apparently constant, despite the large solar-cycle change in surface magnetism, and confirms that this analysis is not sensitive to spurious facular limb-shape contamination. Earlier oblateness measurements have different systematic errors and have yielded marginally larger values. Fivian *et al.* (8), in particular, come close and also have a potentially long time series of measurements to compare with HMI [but see also (18)].

It is, perhaps, surprising that the solar oblateness does not vary with the global solar 11-year cycle, as some of the earlier measurements have suggested (3, 7, 9). We have seen that lower-spatial resolution LDF measurements can introduce a weak correlation between the apparent limb position and brightness (7, 8). Because the limb brightness is intrinsically variable due to faculae, this may affect the apparent solar shape. Our results show that the apparent shape variability is not due to a changing global oblateness. Solar-shape results are now also consistent with a constant solar radius, as measured recently (19), and these results support the helioseismic inference that the solar magnetic cycle does not substantially perturb the interior rotation (by more than $\sim 2\%$) and stratification at low solar latitudes or, on average, in the outer 70% of the solar interior (20).

The dimensionless oblateness coefficient is $C_2 = -5.00 \pm 0.34 \times 10^{-6}$, which is significantly lower in magnitude than the value -5.87×10^{-6} implied by helioseismic rotation data (16). Though this oblateness value is consistent with the early MDI measurement (10), it is now almost 3σ lower than the helioseismic oblateness prediction (16). This difference cannot be accounted for by decreasing the core rotation (for example, in the inner 20% of a solar radius), which is not well constrained by helioseismology. On the other hand, the difference in values could be explained

by a 3 to 10% perturbation in the local rotation rate in the outer few percent of the Sun (16).

Finally, the Sun's mean hexadecapole shape amplitude is small (-0.1 ± 0.4 milli-arc sec) but shows a hint of variability (21). This value is marginally correlated with the sunspot cycle with an amplitude of 2.1 ± 2 milli-arc sec. The hexadecapole shape is also sensitive to the internal solar differential rotation, but if due only to rotation, it would require large changes (on the order of 50%) in the outer parts of the Sun (16) that are not consistent with the constant helioseismic rotation (20) and the constant oblateness. In contrast, solar-cycle changes in near-surface flows or magnetic stresses localized near mid-latitudes could affect C_4 and not the oblateness.

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11. We let $L(r, \theta)$ be the observed LDF function from a binned satellite image. From this, we used the circular average mean LDF represented by $\Gamma(r)$ to solve for a brightness function $\alpha(\theta)$ and the limb shape $\beta(\theta)$. The binned LDF function was then expressed as $L(r, \theta) = [\alpha(\theta) + 1]\Gamma(r - \beta(\theta))$, where α and β represent the mean limb brightness change and position around the limb. We then linearized this equation and solved it as a least-squares problem to find α and β . We obtained the function $\Gamma(r)$ from the binned intensity of limb pixels, whereas we iterated the solution for $\beta(\theta)$ so that $\Gamma(r)$ was adjusted at each iteration by correcting the limb-pixel binning by shifting pixels by the local $\beta(\theta)$ from the previous iteration. This was done for each of the typically 13,000 images obtained during an SDO spacecraft roll. After two iterations, the solution was stable to better than 5%.
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13. Figure S1 shows that the analysis recovers the limb shape, independent of any limb brightness variations. Figure S2 shows that independent simultaneous HMI solar-limb shape and brightness measurements agree on all angular scales and that the limb position and brightness measurements are dominated by solar atmosphere inhomogeneity and its global asphericity.
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15. Figure S3 shows how the limb brightness and position are correlated and how the brightness measurements $[\alpha$, see (11)] can be used to flag localized magnetic limb contamination of the limb shape. The shape analysis is broadly insensitive to the brightness threshold, with no significant change in the derived global oblateness, even with large changes in the assumed brightness threshold.
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21. The χ^2 statistic for describing the hexadecapole amplitude as a constant was 9.4. This was marginally inconsistent (at 95% level) with a constant. Linear regression of the hexadecapole measurements against the sunspot number time series suggested a marginally significant hexadecapole solar-cycle variation with an amplitude of 2.1 ± 2 milli-arc sec.

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Supplementary Materials

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Supplementary Text
Figs. S1 to S3
Table S1
References

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A Physically Transient Form of Silicon Electronics

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A remarkable feature of modern silicon electronics is its ability to remain physically invariant, almost indefinitely for practical purposes. Although this characteristic is a hallmark of applications of integrated circuits that exist today, there might be opportunities for systems that offer the opposite behavior, such as implantable devices that function for medically useful time frames but then completely disappear via resorption by the body. We report a set of materials, manufacturing schemes, device components, and theoretical design tools for a silicon-based complementary metal oxide semiconductor (CMOS) technology that has this type of transient behavior, together with integrated sensors, actuators, power supply systems, and wireless control strategies. An implantable transient device that acts as a programmable nonantibiotic bactericide provides a system-level example.

A n overarching goal in the development of nearly any new class of electronics is to achieve high-performance operation in physical forms that undergo negligible change with time. Active and passive materials, device and circuit layouts, and packaging strategies are each formulated individually and then configured collectively to accomplish this outcome. Here we present concepts and strategies for electronics that involve similar attention to engineering designs, but with the goal of achieving systems that

physically disappear at prescribed times and at controlled rates. Applications that could exploit this transient behavior include implantable medical diagnostic and therapeutic devices that resorb in the body to avoid adverse long-term effects, fieldable environmental sensors that dissolve to eliminate the need for their retrieval, and portable consumer devices that decompose to minimize the costs and health risks associated with recycling and the management of hazardous waste streams. For these three examples, the desired time scales

for transience range from days or weeks, to months, to years, respectively. The approaches reported here can address these and other application concepts with circuit components whose operational characteristics match those of nontransient counterparts formed in the usual way on silicon wafer substrates. When combined with transient sensors, actuators, power supplies, and wireless control systems, this technology provides levels of function that substantially exceed those available

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Fig. 1. Demonstration platform for transient electronics, with key materials, device structures, and reaction mechanisms. (A) Image of a device that includes transistors, diodes, inductors, capacitors, and resistors, with interconnects and interlayer dielectrics, all on a thin silk substrate. (B) Exploded-view schematic illustration, with a top view in the lower right inset. (C) Images showing the time sequence of dissolution in DI water. (D) Chemical reactions for each of the constituent materials with water.

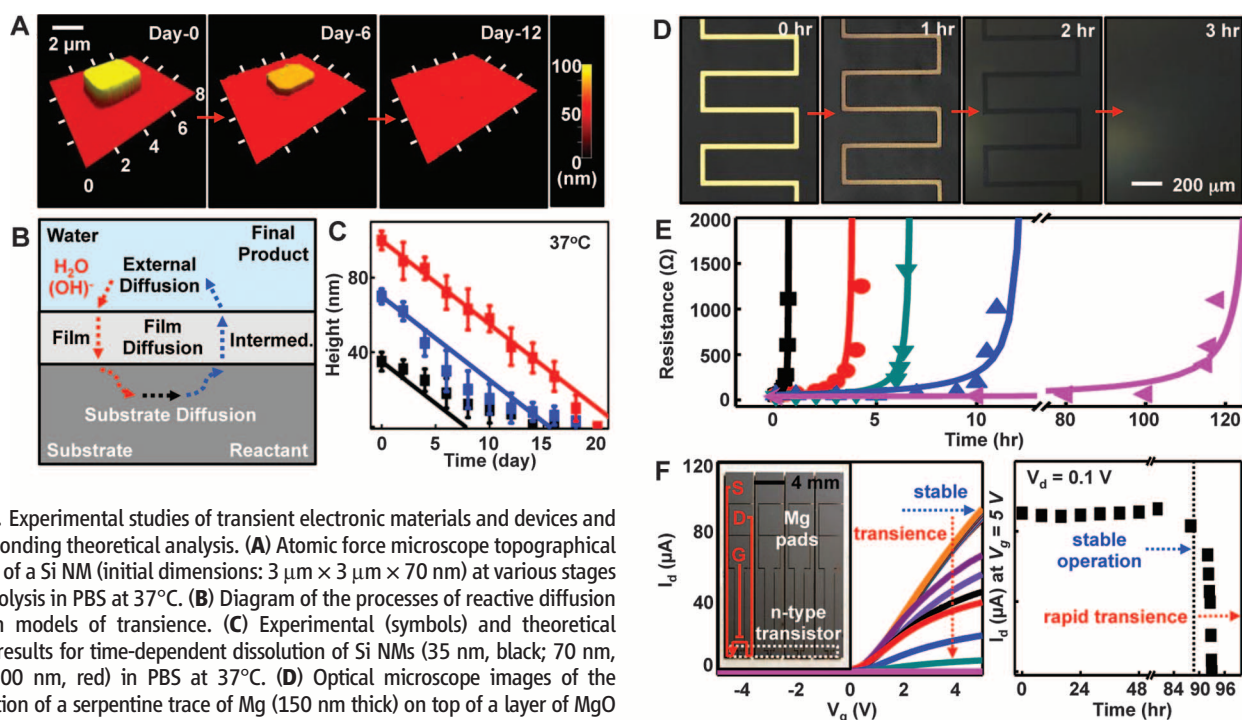
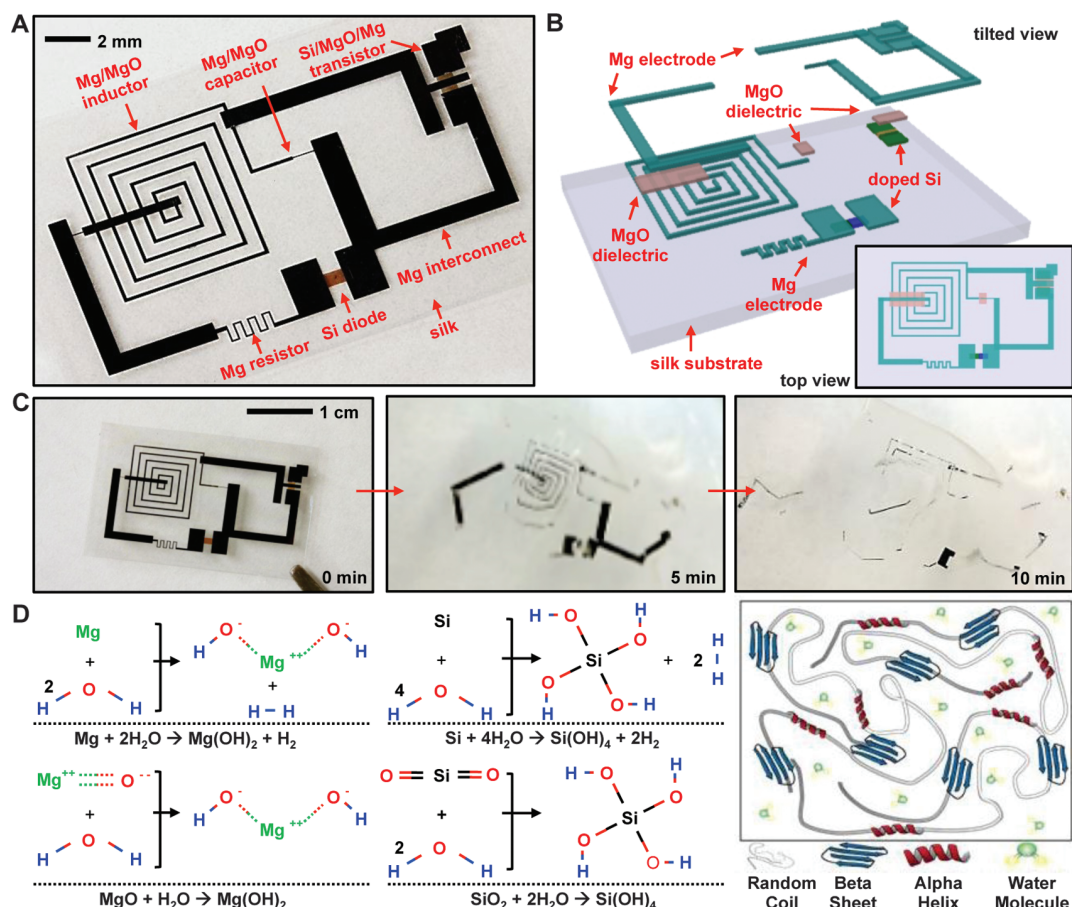


Fig. 2. Experimental studies of transient electronic materials and devices and corresponding theoretical analysis. (A) Atomic force microscope topographical images of a Si NM (initial dimensions: $3\ \mu\text{m} \times 3\ \mu\text{m} \times 70\ \text{nm}$) at various stages of hydrolysis in PBS at 37°C . (B) Diagram of the processes of reactive diffusion used in models of transience. (C) Experimental (symbols) and theoretical (lines) results for time-dependent dissolution of Si NMs (35 nm, black; 70 nm, blue; 100 nm, red) in PBS at 37°C . (D) Optical microscope images of the dissolution of a serpentine trace of Mg (150 nm thick) on top of a layer of MgO (10 nm thick) in DI water at room temperature. (E) Experimental (symbols) and theoretical (lines) results of dissolution kinetics of similar traces of Mg (300 nm thick) with different encapsulating layers: MgO (400 nm, red; 800 nm, blue) and silk (condition i, cyan; condition ii, purple). (F) Measurements of transience in operational characteristics of n-channel transistors

encapsulated by MgO and crystallized silk (picture in the inset on the left) and then immersed in DI water. The results show the drain current (I_d) at $V_d = 0.1\ \text{V}$ as a function of V_g at various times (left) and at $V_g = 5\ \text{V}$ as a function of time (right).

with recently reported forms of organic electronics, in which certain constituent materials are water-soluble (1–3), or with simple nontransient transistors formed on bioresorbable substrates (4).

Figure 1, A and B, and fig. S1 provide images and schematic diagrams of a demonstration platform. All of the components, ranging from the inductors, capacitors, resistors, diodes, transistors, interconnects, and crossovers, to the substrate and encapsulation, disintegrate and dissolve when immersed in deionized (DI) water (Fig. 1C). This example uses magnesium (Mg) for the conductors, magnesium oxide (MgO) (silicon dioxide, SiO_2 , is also possible) for the dielectrics, monocrystalline silicon (Si) nanomembranes (NMs) for the semiconductors, and silk (which is water-soluble and enzymatically degradable) (4, 5) for the substrate and packaging material. The fabrication of systems such as this one involves a combination of transfer printing (Si NMs) (6), physical vapor deposition through fine-line stencil masks (Mg, MgO, and SiO_2), and solution-casting (silk). More details on sample preparation can be found in (6). As adhesion promoters for Mg, we used MgO in certain cases and ultrathin layers of Ti in others. Device yields without the Ti are 70 to ~80% with evaporated Mg and >90% with sputtered Mg.

The chemical reactions responsible for the dissolution of each material appear in Fig. 1D. The Si NMs and layers of SiO_2 are particularly important because of their essential roles in high-performance transistors, diodes, photodetectors, solar cells, temperature sensors, strain gauges, and other semiconductor devices. The NM geometry is critical because it enables high-performance devices and planar architectures, minimizes the amount of material that must be consumed during the transient step, and provides mechanics and processing options that are favorable for heterogeneous integration onto substrates such as silk (4), as well as elastomers that can provide modulus-matched interfaces with the body (7). A typical transistor described here requires less than ~1 μg of Si, which can be dissolved in as little as 30 μl of biofluid (8).

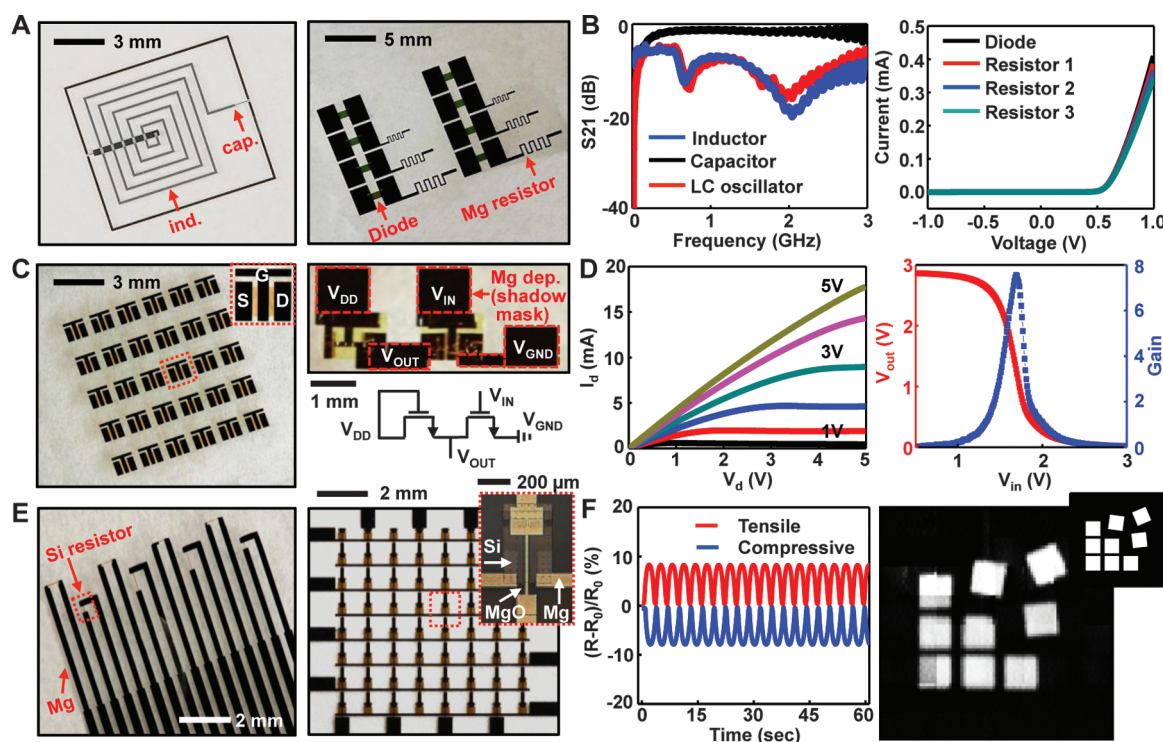
Figure 2A presents atomic force micrographs of a Si NM ($3 \times 3 \mu\text{m}$) with a thickness of 70 nm, collected at different stages of dissolution in phosphate-buffered saline (PBS; pH of 7.4) at 37°C, to simulate transience by bioresorption (see figs. S2 and S3A for additional data). The dissolution involves hydrolysis to form $\text{Si}(\text{OH})_4$ (9), according to $\text{Si} + 4 \text{H}_2\text{O} \leftrightarrow \text{Si}(\text{OH})_4 + 2 \text{H}_2$, where SiO_2 can sometimes be involved as an intermediate (10). The simplest model of the

kinetics, which depends strongly on pH, considers a constant reaction rate at the water/Si NM interface (11). The results capture experimental observations at both body temperature (37°C) (Fig. 2C) and room temperature (25°C) (fig. S3A) for a dissolution rate of 4.5 nm/day and 2 nm/day, respectively, consistent with Arrhenius scaling (12).

Mechanisms involving diffusion into the materials can be important for Mg and MgO deposited by electron-beam evaporation and SiO_2 formed by chemical vapor deposition, or as an intermediate in the hydrolysis of Si. In such cases, the kinetics can be described analytically using models of reactive diffusion (Fig. 2B) (6). The results quantitatively account for related behaviors in other materials for transient electronics, including those in Fig. 1 (6). Figure 2D presents a meander trace of Mg (150 nm) on a thin film of MgO (10 nm; adhesion promoter), in which the measured changes in resistance correlate well with those expected based on computed changes in thickness (Fig. 2E and fig. S4, A and B) (6). (Other examples appear in fig. S5.) This result connects a key electrical property to models of reactive diffusion, thereby suggesting the capacity to use such analytics in conjunction with established circuit simulators as a comprehensive design approach.

Fig. 3. Images and electrical properties of transient electronic components, circuits, and sensors, including simple integrated circuits and sensor arrays. (A) Image of an LC (inductor-capacitor) oscillator fabricated with Mg electrodes and MgO dielectric layers (left) and an array of Si NM diodes with serpentine Mg resistors (right). (B) Measurements of the S21 scattering parameter of an inductor (blue), capacitor (black), and LC oscillator (red) at frequencies up to 3 GHz (left). Current-voltage (I - V) characteristics of diodes connected to three different Mg resistors (right) are shown. (C) Images of an array of p-channel (left) MOSFETs and a logic gate (inverter; right) composed of n-channel MOSFETs.

The MOSFETs use Mg source (S), drain (D), and gate (G) electrodes; MgO gate dielectrics; and Si NM semiconductors. The inverter uses Mg for interconnects and Au for source, drain, and gate electrodes, in a circuit configuration shown in the diagram. (D) I - V characteristics of a representative n-channel MOSFET [left, channel length (L_{ch}) and width (W) are 20 μm and 900 μm , respectively]. Transfer characteristic for the inverter (right, L_{ch} and W are 20 μm and 700 μm for the input transistor and 500 μm and 40 μm for the load transistor, respectively). The voltage gain is ~8. (E) Image of



strain sensors based on Si NM resistors (left) and an addressable array of Si NM photodetectors with blocking diodes. In both cases, Mg serves as contact and interconnection electrodes and MgO as the dielectric. (F) Fractional change in resistance of a representative strain gauge as a function of time during cyclic loading (left). R , bent; R_0 , flat. Bending induces tensile (red) and compressive (blue) strains uniaxially up to ~0.2%. Right, image of a logo collected with the photodetector array. The inset shows the logo design.

The transience times for NM-based electronic components can be extended, in controlled amounts, by adding transient encapsulating layers and packaging materials; they can be reduced by decreasing the critical dimensions or by physically structuring the materials in a way that accelerates dissolution by disintegration (fig. S6). Figure 2E and fig. S4 show results of measured transience in a serpentine resistor of Mg, encapsulated with different thicknesses of MgO and with combinations of MgO and overcoats of silk. Corresponding modeling results are also shown in (6). Silk is attractive for this purpose because its solubility in water can be programmed, over several orders of magnitude, through the control of crystallinity (5, 13). Other biodegradable polymers can also be used, as shown in fig. S7.

Studies of transience at the device level are also important. Figure 2F shows examples of metal oxide semiconductor field-effect transistors (MOSFETs) formed using Si NMs, SiO₂ gate dielectrics, and Mg electrodes, with encapsulating layers of MgO and crystallized silk. The devices

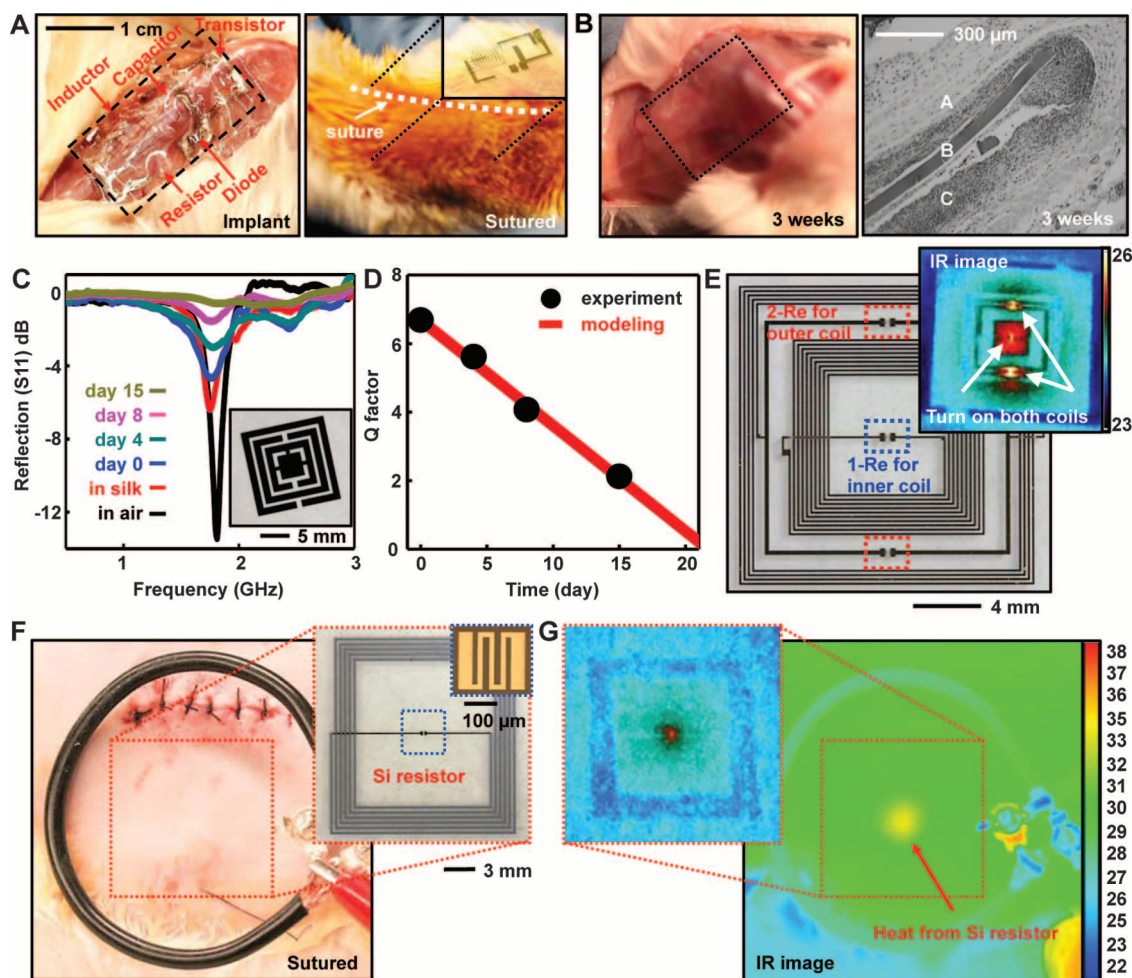
show two-stage kinetics in their functional transience. Immersion in DI water for up to ~90 hours causes negligible change in key device characteristics. Functional degradation then occurs in a relatively narrow time interval after this period of stable operation. The encapsulation defines the first time scale; the Mg electrodes define the second. The results demonstrate that the transience time can be engineered in a way that is decoupled from system- or device-level function.

These materials, fabrication techniques, and modeling tools can yield components for almost any type of transient electronic system, in CMOS designs. Figure 3 presents several examples, including additional details on MOSFETs similar to those in Fig. 2F, where both n- and p-channel operation is possible. The resulting electrical properties for an n-channel device include saturation and linear regime mobilities of 560 cm²/V·s and 660 cm²/V·s, respectively, on/off ratios of >10⁵, subthreshold slopes of 160 mV/dec [at drain voltage (V_d) = 0.1 V] and width-normalized current outputs of 0.34 mA/mm [at gate voltage

(V_g) = 5 V]. These characteristics, as well as those of similar p-channel devices, compare favorably to the performance of counterparts formed on Si-on-insulator (SOI) wafers (14). [For the range of channel lengths investigated, contact resistances do not limit performance (fig. S8).] In all cases, the transience times of different elements in an integrated system can be controlled by use of varied thicknesses and/or stack compositions, or even via combination with nontransient materials. This last possibility is shown in a logic gate (inverter) in the righthand panels of Fig. 3, C and D, where a nontransient metal (Au) serves as source, drain, and gate electrodes for two transistors joined by transient Mg interconnects.

Many other classes of semiconductor devices and passive components are possible, with examples in Fig. 3 and figs. S9 and S10. The resistors and diodes can serve as temperature sensors; the latter can also be used in photodetectors and solar cells, as shown in Fig. 3 and fig. S10. The Si NM diode and Mg resistive temperature sensors show sensitivities of ~2.23 mV/°C (change in

Fig. 4. In vivo evaluations and example of a transient bioresorbable device for thermal therapy. **(A)** Images of an implanted (left) and sutured (right) demonstration platform for transient electronics located in the subdermal dorsal region of a BALB/c mouse. **(B)** Implant site after 3 weeks (left). (Right) Histological section of tissue at the implant site, excised after 3 weeks, showing a partially resorbed region of the silk film. (A, subcutaneous tissue; B, silk film; C, muscle layer). **(C)** Resonant responses of an implanted transient rf meta-material structure before and after placement in a silk package, immediately after implantation and at several time intervals thereafter. **(D)** Measured and calculated Q factor for the metamaterial. The results indicate transience dominated by the diffusion of biofluids through the silk package. **(E)** Transient wireless device for thermal therapy, consisting of two resistors (red outline) connected to a first wireless coil (70 MHz; outer coil) and a second resistor (blue outline) connected to a second, independently addressable, wireless coil (140 MHz; inner coil). The inset shows a thermal image of this device coupled with a primary coil operating at two frequencies, to drive both the inner and outer coils simultaneously. **(F)** Primary coil next to a sutured implant site for a transient thermal therapy



device. The inset shows an image of a device. **(G)** Thermal image collected while wirelessly powering the device through the skin; the results show a hot spot (5°C above background) at the expected location, with a magnified view in the inset.

voltage for a given current output) and 0.23%/°C (percentage change in resistance), both of which are consistent with the behavior of conventional, nontransient devices (15). Ultrathin Si solar cells (~3 μm thick) provide fill factors of 66% and overall power conversion efficiencies of ~3%, even without light-trapping structures, backside reflectors, or antireflection coatings. Doped Si NMs can serve as strain gauges (Fig. 3E, left), with gauge factors of nearly ~40 (Fig. 3F, left, and fig. S10B), which are comparable to those of state-of-the-art devices (16). As an example of interconnected components, we built a transient digital imaging system, consisting of collections of Si NM photodiodes with blocking diodes for passive matrix addressing (Fig. 3E, right), capable of capturing pictures when operated in a scanned mode (Fig. 3F, right, and fig. S10D). (See more details on device dimensions in fig. S11.) The yield here is >90% [i.e., 58 out of 64 pixels were fully functional (fig. S12)]. One possibility for power supply involves Si solar cells such as those shown in fig. S10A. Another uses inductors and capacitors like those in Figs. 1A and 3A and fig. S9 as wireless antennas for near-field mutual inductance coupling to separately powered, external primary coils. This option is interesting for implantable devices (4), made possible by the biocompatibility of the constituent materials (Fig. 1), as established in unrelated contexts (6).

To demonstrate opportunities, we conducted a series of in vivo and in vitro experiments. Various representative transient devices were fabricated, sealed in silk packages, sterilized with ethylene oxide, and then implanted in the subdermal region of BALB/c mice in accordance with Institutional Animal Care and Use Committee protocols. Figure 4A shows the case of the platform in Fig. 1. Examination after 3 weeks (Fig. 4B, left) revealed only faint residues, with evidence of slow reintegration into the subdermal layers, along with apparent revascularization. The histological section in Fig. 4B (right) shows the subdermal layer (A), the silk film (B), and the muscle layer (C) and reveals no significant inflammatory reactions. Additional analysis appears in fig. S13.

Inductive coils of Mg combined with resistive microheaters of doped Si NMs, integrated on silk substrates and housed in silk packages, can provide transient thermal therapy to control surgical site infections (17, 18) as a nonantibiotic, programmable bacteriocidal appliqué that disappears as the patient moves beyond the period of greatest risk. In vitro tests demonstrate the efficacy of this approach (6). Figure 4, C and D, present a metamaterial rf antenna, as a generalized component for such a device, capable of continuous wireless monitoring after implantation. The data indicate transient behavior associated with the slow diffusion of biofluids through the edges of the silk package, with a measured quality (Q) factor that has time dependence consistent with theoretical models (6). Figure 4E shows an image of a functional device formed

on glass that includes two coils with different resonance frequencies (~70 and ~140 MHz) and three separate heaters. Wirelessly operating either or both of these coils with appropriate frequencies and power levels applied to a separate primary coil enables full control of the system, as illustrated in the thermal image in the inset. (See figs. S14 to S16 for other examples.) To illustrate in vivo functionality, a fully transient version of this device was implanted under the skin of a Sprague-Dawley rat (Fig. 4F). Inductive coupling through the skin generates a localized temperature increase of $\Delta T \sim 5^\circ\text{C}$ (Fig. 4G), coincident with the position of the heater. The functional transience has a time scale of 15 days, chosen via the crystallinity of the silk, to coincide with the most critical period, which is the first few days after an operation, to sterilize and maintain asepsis at the wound site. After this time, the device disappears, leaving only remnants of silk, which resorb on longer time scales, to eliminate the long-term burden associated with additional exogenous implant material.

Concepts reported here establish a baseline of materials, modeling approaches, manufacturing schemes, and device designs for transient electronic systems, sensors, actuators, and power supplies. The Si NMs are critically important elements, because their use enables sophisticated semiconductor components with both active and passive functionality. For the dielectrics and conductors, additional possibilities range from collagen to poly(lactic-co-glycolic acid) and from iron to zinc, respectively. Alternative modes of transience include absorption, corrosion, and depolymerization. The rates for these processes could, conceivably, be adjustable in real time and/or sensitive to the properties of the surrounding environment, determined by chemical or biological events, or changes in temperature, pressure, or light. Combining such possibilities in transience with ideas in soft, tissue-like electronics will further expand opportunities for applications in biomedical devices (7).

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Supplementary Materials

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Gold-Catalyzed Direct Arylation

Liam T. Ball, Guy C. Lloyd-Jones,* Christopher A. Russell*

Biaryls (two directly connected aromatic rings, $\text{Ar}^1\text{-Ar}^2$) are common motifs in pharmaceuticals, agrochemicals, and organic materials. Current methods for establishing the $\text{Ar}^1\text{-Ar}^2$ bond are dominated by the cross-coupling of aryl halides ($\text{Ar}^1\text{-X}$) with aryl metallics ($\text{Ar}^2\text{-M}$). We report that, in the presence of 1 to 2 mole percent of a gold catalyst and a mild oxidant, a wide range of arenes ($\text{Ar}^1\text{-H}$) undergo site-selective arylation by arylsilanes ($\text{Ar}^2\text{-SiMe}_3$) to generate biaryls ($\text{Ar}^1\text{-Ar}^2$), with little or no homocoupling ($\text{Ar}^1\text{-Ar}^1/\text{Ar}^2\text{-Ar}^2$). Catalysis proceeds at room temperature and tolerates a broad range of functional groups, including those incompatible with cross-coupling. These features expedite biaryl preparation, as demonstrated by synthesis of the nonsteroidal anti-inflammatory diflunisal.

The biaryl moiety (two directly connected aromatic rings, $\text{Ar}^1\text{-Ar}^2$) is a common functionality in pharmaceuticals [such as Lipitor, Crestor, and Diovan, three of the

most widely prescribed drugs in 2010 (1)]; in agrochemicals; and in many modern organic materials, including liquid crystal displays, light-emitting diodes, and conducting polymers. The

high value of the biaryl motif is reflected in the myriad strategies for its construction, the majority of which involve transition metal-catalyzed cross-coupling of an aryl halide ($\text{Ar}^1\text{-X}$) and an aryl organometallic ($\text{Ar}^2\text{-M}$) (Fig. 1) (2, 3). Nonetheless, it is widely appreciated that there remains a need for biaryl syntheses that are more concise, more selective, more versatile, or complementary to conventional routes. In response to this need, much effort has been devoted to direct arylation: cross-couplings in which one preactivated partner is replaced by a simple arene [for reviews, see (4–7); for selected examples, see (8–14)].

Direct arylation of an arene ($\text{Ar}^1\text{-H}$) with $\text{Ar}^2\text{-M}$ constitutes coupling of two nominal nucleophiles and hence requires an oxidant. As a corollary of the oxidative mechanism, functional groups incompatible with traditional cross-coupling may be tolerated; for example, when one or both partners bear additional (pseudo)halogens (i.e., in Fig. 1, FG^1 and/or $\text{FG}^2 = \text{X}^2, \text{X}^3$). The mechanistically orthogonal direct arylation approach then facilitates a powerful synthetic strategy for step-economic construction of complex biaryl molecules; for example, delivering versatile halogenated biaryl systems primed for direct entry into the cross-coupling manifold. Although tremendous progress has been made in the field of (oxidative) direct arylation (4–14), delivery of an efficient protocol remains nontrivial. In particular, the inherent low reactivity of a C-H bond (relative to the C-X bond it replaces) must be overcome, and high selectivity must be exhibited for a specific C-H bond in $\text{Ar}^1\text{-H}$, without competition from C-H bonds in the coupling partner ($\text{Ar}^2\text{-M}$) or the product ($\text{Ar}^1\text{-Ar}^2$). Many of the extant methodologies involve harsh reaction conditions, limiting the substrate scope, and employ high catalyst loadings. Moreover, selectivity is frequently achieved by the use of an excess of one coupling partner (for example, as the solvent), and/or substrates incorporating a directing group capable

of delivering the transition-metal catalyst to a specific C-H bond (15).

We report a gold-catalyzed direct arylation of simple arenes ($\text{Ar}^1\text{-H}$) with arylsilanes ($\text{Ar}^2\text{-SiMe}_3$) that affords biaryls ($\text{Ar}^1\text{-Ar}^2$) under remarkably mild conditions. The site of arylation is predictable and not dependent on the presence of adjacent, coordinating functionality. Both partners (Ar^1 and Ar^2) can be decorated with a diverse array of functionality, including halogens, thus providing valuable biaryl building blocks.

The stimulus for pursuing the direct arylation described here was provided by two recently disclosed gold-catalyzed processes: the dehydrogenative homocoupling of $\text{Ar}^1\text{-H}$ in the presence of iodine(III), as reported by Tse (16, 17), and a competing homocoupling of $\text{Ar}^2\text{-SiMe}_3$ that we observed in gold-catalyzed oxidative additions to alkenes (18, 19). Although these reactions do generate biaryls, they are by definition symmetrical products ($\text{Ar}^1\text{-Ar}^1$ and $\text{Ar}^2\text{-Ar}^2$) and thus of limited value. Arylsilanes are well documented to react via electrophilic aromatic substitution pathways ($\text{S}_{\text{E}}\text{Ar}$), analogously to simple arenes, with ipso substitution of the silyl moiety rather than loss of a proton (20). We thus hypothesized that $\text{S}_{\text{E}}\text{Ar}$ might be operative in both homocoupling processes. Extension of this argument raised the tantalizing possibility that site selective gold-catalyzed oxidative heterocoupling of arylsilanes with arenes (to give $\text{Ar}^1\text{-Ar}^2$) should provide a pathway for a synthetically valuable direct arylation. The feasibility of this process was confirmed by a ^{19}F nuclear magnetic resonance (NMR) study of mixtures of mesitylene (1,3,5-trimethylbenzene, $\text{Ar}^1\text{-H}$) with (4-fluorophenyl)trimethylsilane ($\text{Ar}^2\text{-SiMe}_3$) in the presence of a gold(I) precatalyst (Ph_3PAuCl) and an iodine(III) oxidant at 65°C . The desired direct arylation process was detected but accompanied by homocoupling of the arylsilane ($\text{Ar}^2\text{-Ar}^2$), a competing electrophilic substitution of $\text{Ar}^1\text{-H}$ by iodine(III), and further arylation of the biaryl product ($\text{Ar}^2\text{-Ar}^1\text{-Ar}^2$).

Four criteria were identified as key for the delivery of a practical and general direct arylation process, leading us to engage in an extensive program of reaction refinement (21): (i) high selectivity for heterocoupling over homocoupling; (ii)

economic, ideally stoichiometric, quantities of the reactants; (iii) tolerance of a wide range of functionalities; and (iv) efficient catalysis at convenient temperatures, without the need for inert atmospheres.

Using these criteria as guiding principles, conditions were developed that facilitate high-yielding direct arylation, in the majority of cases with near-complete suppression of all side reactions (Tables 1 and 2). Key developments were using Ph_3PAuOTf as a precatalyst (table S2), conducting the reaction in the presence of a low concentration of methanol co-solvent at room temperature (tables S3 and S4), and forming the active oxidant in situ from iodobenzene diacetate [$\text{PhI}(\text{OAc})_2$] and camphorsulfonic acid (CSA) (tables S1 and S4); the latter are both commercially available, bench-stable free-flowing solids. Control reactions established the essential role of a gold precatalyst over other late transition elements or Brønsted/Lewis acid promoters (table S5) and demonstrated both the innocence of diaryliodonium salt side-products and the absence of uncatalyzed arylation, even at elevated temperatures (schemes S1 and S2).

Two general sets of conditions were identified, which were tailored to the cost and complexity of the arene: for heavily functionalized or valuable arenes, a 1:1 stoichiometry of coupling partners was used (conditions A), whereas for simple or cheap arenes, a 2:1 stoichiometry was used and the precatalyst loading reduced from 2 to 1 mole % (mol %) (conditions B). Most reactions proceeded to completion within 20 to 40 hours at room temperature, although some were notably quicker (21). Longer reaction times (up to 80 hours) or higher temperatures (up to 65°C) were required when reactants were sterically hindered (14, 31, and 32), or when less electron-rich arenes (4, 8, 20, and 28) or electron-deficient arylsilanes (46 and 47) were coupled, these reactivity trends being consistent with $\text{S}_{\text{E}}\text{Ar}$ elementary steps for both partners. The arylation process exhibits excellent site selectivity with respect to the arene partner ($\text{Ar}^1\text{-H}$, Table 1), the position of arylation also being readily predictable based on the well-understood patterns of $\text{S}_{\text{E}}\text{Ar}$ (20). The mildness of the conditions is evident from the

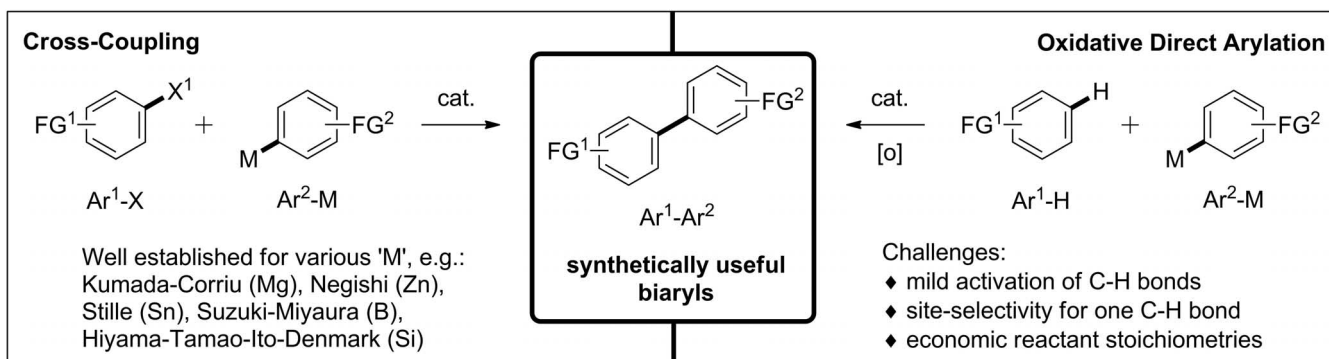


Fig. 1. Cross-coupling and oxidative direct arylation approaches to biaryl ($\text{Ar}^1\text{-Ar}^2$) building blocks. X^1 , halide or sulfonate; M, electropositive element (such as Mg, Zn, B, Sn, or Si); $\text{FG}^{1,2}$, functional group (including alkyl, aryl, halogen etc); [O], oxidant; cat., transition-metal catalyst.

Table 1. Scope of Ar¹-H in Au-catalyzed direct arylation. Conditions: arylsilane (0.50 mmol), PhI(OAc)₂ (0.65 mmol), and CSA (0.75 mmol) in CHCl₃/CH₃OH (50:1) at room temperature, and **A**, Ar¹-H (0.50 mmol) and

Ph₃PAuOTs (2 mol %); or **B**, Ar¹-H (1.00 mmol) and Ph₃PAuOTs (1 mol %). Ts, *p*-toluenesulfonyl; Ms, methanesulfonyl; Piv, pivaloyl (COCMe₃); NPhth, *N*-phthalimido.

Conditions A.			Conditions B.		
	1 X = Cl	85%		17	68% #
	2 X = Br	92%		18	63% **
	3 X = I	88%		19	46% ††
	4 X = OMs	81% *		20	93% ††
	5 X = CO ₂ Me	92%		21	82% §§
	6 X = NMePiv	94%		22	71%
	7 X = NPhth	88%		23	74%
	8	65% †, ‡		24	74%
	9	59% §		25	65%
	10 X = OH	82%		26	78% *
	11 X = OMs	71%		27	57%
	12 X = OCON ⁱ Pr ₂	69%		28	37% †, ¶¶
	13 X = NPhth	83%		29	6% †, ‡, ###
	14	74%		30	0 †
	15	91%			
	16	29% ¶¶			

*0.75 mmol Ar¹-H. †IBA (1-hydroxy-1,2-benzodioxol-3-(1*H*)-one) replaces PhI(OAc)₂ at 65°C. ‡Yield by ¹H/¹⁹F NMR. §1.5 mol % Au. ||With X = OSiⁱPr₃, in situ deprotection yields **10** (63%). ¶¶Site selectivity: 57%, with 2,5-diarylated thiophene (17%) also formed. §§Site selectivity: 89%. **2,6-Diarylated anisole (10%) also formed. ††Site selectivity: 67%, with diarylated anisole also detected (8%, by gas chromatography–mass spectrometry). ‡‡Site selectivity: 80%. §§Site selectivity: 87%. ||0.50 mmol Ar¹-H. ¶¶4,4'-Difluorobiphenyl (homocoupling product, 37%) and 1,4-diarylated benzene (10%) also formed. ###4-(Bromophenyl)trimethylsilane replaces 4-(fluorophenyl)trimethylsilane, Ar^{Br} = 4-bromophenyl.

reaction of (4-fluorophenyl)trimethylsilane with a range of ortho-anisole derivatives under conditions **A**, to give the corresponding biaryls in high yield and with excellent site selectivity (97 to 99% isomeric purity) (**21**).

A wide range of functional groups is tolerated, including synthetically useful (pseudo)halogenated species (**1** to **4**), esters and amides (**5** to **8**), and sensitive functionalities remote from the ring, such as primary alcohols (**9** and **10**) and sulfonate (**11**). Biaryl **8** also demonstrates tolerance of an amine in the gold-catalyzed direct arylation; a basic nitrogen moiety is present in all of the 10 most widely prescribed drugs of 2010 (*1*). In compounds **1** to **8**, **14**, and **17** to **19**, the methoxy substituent itself is of further synthetic potential; for example, di-

recting S_EAr and ortho-metallation, or via derivatization, cross-coupling, amination, or reductive cleavage [for a review of catalytic activation of arylmethyl ethers, see (**22**)].

Simple arenes, including those lacking strongly electronically activating and/or directing groups, also proved excellent substrates and were ideal for reaction under conditions **B**; the site selectivity observed with anisole and toluene, giving biaryls **17** and **20**, respectively, being consistent with S_EAr by a gold(III) electrophile (**23**). Although the electron-rich 4-methylanisole, in which the position para to the methoxy substituent is blocked, promoted double arylation, **18** was generated with >99.7% discrimination between electronically different sites. In contrast, 3-methylanisole, in which

the 4- and 6- positions are similarly activated, underwent arylation to give **19** as a 2:1 mixture of isomers. Arylation even occurred smoothly at C-H bonds flanked on either side by methyl substituents (**24** to **26**), and although the parent naphthalene reacted with low selectivity (**21**), the methyl-substituted analog generated biaryl **27** with >96% site selectivity, in just 5 hours at room temperature.

A preliminary investigation indicated that thiophenes are viable substrates, although, without further optimization, the conditions proved less general [for example, compare **15** (91%, >99.5% site selective) and **16** (29%, 57% site selective)]. The low reactivity of electron-deficient (hetero) arenes, such as PhF (**29**) and pyridine (**30**), is

Table 2. Scope of Ar²-SiMe₃ in Au-catalyzed direct arylation. Reaction conditions are as in Table 1. Tf, trifluoromethanesulfonyl.

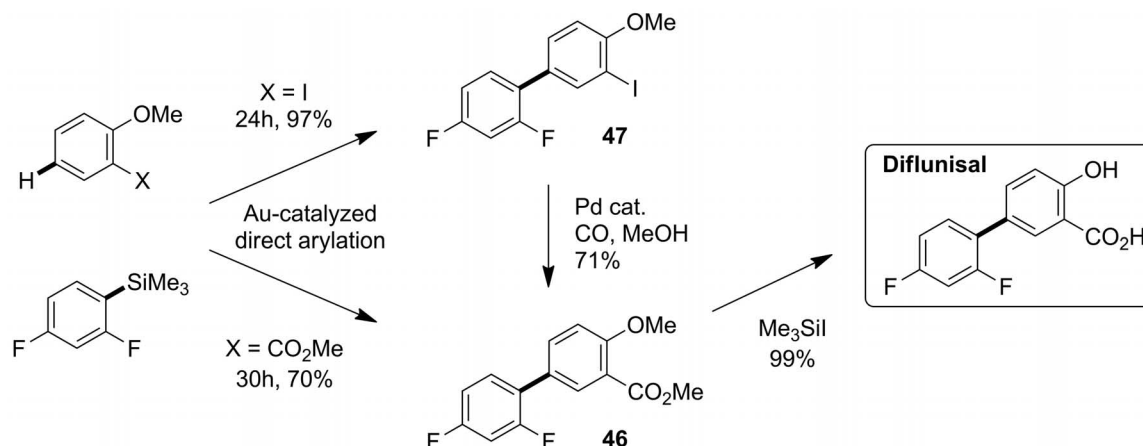
Ar ² -SiMe ₃	Ar ¹ -H	Ar ² -Ar ¹																																																		
				<table><tr><td>31</td><td>R = 2-F</td><td>92% *</td><td>39</td><td>R = 4-CO₂Me</td><td>70%</td></tr><tr><td>32</td><td>R = 2-OMs</td><td>70% *</td><td>40</td><td>R = 4-OTf</td><td>72% †</td></tr><tr><td>33</td><td>R = 4-Cl</td><td>97%</td><td>41</td><td>R = 4-OPiv</td><td>80%</td></tr><tr><td>34</td><td>R = 4-Br</td><td>91%</td><td>42</td><td>R = 4-NMePiv</td><td>59% ‡, §</td></tr><tr><td>35</td><td>R = 4-I</td><td>67%</td><td>43</td><td>R = 4-NMeTs</td><td>91%</td></tr><tr><td>36</td><td>R = 3-Br</td><td>72%</td><td>44</td><td>R = H</td><td>84% ‡</td></tr><tr><td>37</td><td>R = 3-CHO</td><td>77%</td><td>45</td><td>R = 4-Me</td><td>55% *, </td></tr><tr><td>38</td><td>R = 4-Piv</td><td>90%</td><td></td><td></td><td></td></tr></table>	31	R = 2-F	92% *	39	R = 4-CO ₂ Me	70%	32	R = 2-OMs	70% *	40	R = 4-OTf	72% †	33	R = 4-Cl	97%	41	R = 4-OPiv	80%	34	R = 4-Br	91%	42	R = 4-NMePiv	59% ‡, §	35	R = 4-I	67%	43	R = 4-NMeTs	91%	36	R = 3-Br	72%	44	R = H	84% ‡	37	R = 3-CHO	77%	45	R = 4-Me	55% *,	38	R = 4-Piv	90%			
31	R = 2-F	92% *	39	R = 4-CO ₂ Me	70%																																															
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38	R = 4-Piv	90%																																																		

*IBA replaces PhI(OAc)₂ at 65°C.

†1 mol % Au.

‡Using conditions B.

§Arylsilane homocoupling product (29%) also formed.

||1.00 mmol Ar¹-H, arylsilane homocoupling**Fig. 2.** Diflunisal via gold-catalyzed direct arylation; yields are unoptimized. Site selectivity for the arylation step (**46** and **47**) is 98 to 99%.

again consistent with an S_EAr mechanism. The selectivity for electron-rich aromatics not only suppresses over-arylation but is also complementary to direct arylation processes that proceed via deprotonation-type mechanisms and favor electron-poor substrates or require ortho-directing groups (4–7, 15).

Diverse functionality could also be introduced through the arylating partner (Ar²-SiMe₃, Table 2). Ortho-substituted arylsilanes that reacted sluggishly at room temperature gave the corresponding biaryls (e.g., **31** and **32**) in excellent yield at 65°C, using a less reactive iodine(III) oxidant. The synthetically useful halogens and sulfonates (**31** to **36** and **40**) were well tolerated, as were an aldehyde (**37**) and a pivaloyl ester (**41**) that remained in the products without oxidation or transesterification, respectively. Although electron-rich silanes proved more challenging, as a result of arylsilane homocoupling, the selection of appropriate reaction conditions allowed even biaryl **45** to be isolated in reasonable yield. Homocoupling of the arene partner (16, 17) was not observed; instead, the material balance of the less efficient reactions detailed in Table 1 (typically for electron-rich Ar¹-H) comprised diarylation and diaryliodonium side products (21).

The utility of the direct arylation methodology is exemplified by the preparation of diflunisal (Dolobid, Merck & Co.), a non-opioid, nonsteroidal anti-inflammatory drug (24, 25). Unoptimized direct arylation of methyl ortho-anisate (X = CO₂Me,

Fig. 2) with (2,4-difluorophenyl)trimethylsilane afforded the corresponding biaryl **46**; routine demethylation gave diflunisal (69%). Previous methods for forging the key biaryl linkage of diflunisal include Gomberg-Bachmann coupling of a diazonium salt and traditional cross-coupling (21). The power of the direct arylation approach is also evident in a second route to diflunisal, proceeding via biaryl **47**; this iodide intermediate can also serve as a versatile platform for structural diversification via a broad spectrum of stoichiometric and catalytic methodologies, including cross-coupling.

Compared to many other direct arylations (4–14), gold-catalyzed arylation with arylsilanes is notable for its mild conditions, low loadings, and site selectivity. It also provides complementarity and orthogonality to traditional cross-coupling strategies (2, 3), allowing the strategic linking of the two processes (Fig. 2). Arylsilanes are particularly valuable as substrates, because the stability of the silyl moiety allows it to be installed early in a synthesis, and they are readily handled, lacking the toxicity and/or air sensitivity that can be displayed by the aryl metallics traditionally used in cross-coupling. Their synthesis, via silylation of a range of precursors that includes arenes, aryl halides, benzonitriles, and arylmetallics, is generally straightforward (21), and both camphorsulfonic acid and iodobenzene diacetate are commercially available; alternatively, the latter can be very efficiently prepared from iodobenzene with a cheap

stoichiometric oxidant (21). In addition, and in contrast to such metals as palladium and nickel, gold residues are regarded as relatively benign, and the Ph₃PAuOTs precatalyst can be prepared in near-quantitative yield (26) in a single step from commercially available precursors. All of these aspects suggest the expedient application of gold-catalyzed direct arylation for the concise construction of synthetically valuable biaryl building blocks for pharmaceutical, agrochemical, and materials chemistry.

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Supplementary Materials

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Synthesis and Characterization of a Rhodium(I) σ -Alkane Complex in the Solid State

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Transition metal–alkane complexes—termed σ -complexes because the alkane donates electron density to the metal from a σ -symmetry carbon–hydrogen (C–H) orbital—are key intermediates in catalytic C–H activation processes, yet these complexes remain tantalizingly elusive to characterization in the solid state by single-crystal x-ray diffraction techniques. Here, we report an approach to the synthesis and characterization of transition metal–alkane complexes in the solid state by a simple gas-solid reaction to produce an alkane σ -complex directly. This strategy enables the structural determination, by x-ray diffraction, of an alkane (norbornane) σ -bound to a d⁸–rhodium(I) metal center, in which the chelating alkane ligand is coordinated to the pseudosquare planar metal center through two σ -C–H bonds.

The recognition that the σ -bond of dihydrogen, or the C–H bond of an ancillary ligand (an intramolecular agostic interaction), can interact with a metal center to form a so-called σ -complex was a landmark discovery in transition metal chemistry (1). In such complexes, the C–H (or H–H) bonds serve as ligands to the metal center through donation of their σ -bonding electron pair. The chemistry of such species is now well established. In contrast, the synthesis of transition metal–alkane σ -complexes (Fig. 1) (1–3), key intermediates in C–H activation processes (4–6), represents one of the major challenges for organometallic chemists over the past 40 years. Although the first examples were suggested by spectroscopic data arising from matrix isolation experiments at 12 K (7), σ -complexes

remain tantalizingly elusive species to characterize fully, especially compared with the closely related σ -complexes of H₂ (1) or agostic complexes (8). The existence of σ -complexes as intermediates is often inferred from detailed kinetic studies of C–H activation processes (9), whereas direct observation relies on solution spectroscopic techniques such as fast time-resolved infrared spectroscopy (10, 11) or nuclear magnetic resonance (NMR) spectroscopy (12), generally at low tem-

peratures (for example, at –83°C). Important recent advances include characterization of relatively long-lived (in hours) σ -alkane complexes in solution for a variety of simple alkanes using Mn- or Re-based metal fragments (13, 14), as well as a methane σ -complex at a d⁸–rhodium(I) center (Fig. 1A) (15). This complex, stable in CDCl₃ solution at –110°C, is formed directly by protonation of a Rh–CH₃ bond.

Only two examples have been reported in which a saturated hydrocarbon has been located within the coordination sphere of a metal center in the solid state, allowing for an analysis of the molecular structure by single-crystal x-ray diffraction techniques. The first of these (Fig. 1B) shows a molecule of heptane interacting with an iron(II)–porphyrin complex (16). Unfortunately, crystallographic disorder prevented the accurate analysis of the Fe–alkane interaction. The second example (Fig. 1C) involves cyclic alkane adducts of an unsaturated uranium(III) complex (17). Both structures in Fig. 1, B and C, result from incorporation of a solvent molecule within the coordination sphere of the metal, potentially assisted by host-guest effects in addition to the direct C–H...M bond interaction (here, M represents a metal). Neither of these complexes are stable on solvation. Very recently, the binding of light alkanes to iron centers in an extended metal-organic framework was characterized by powder

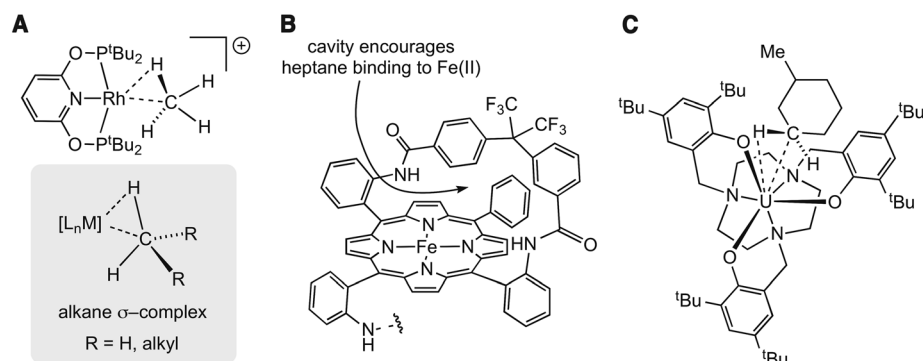


Fig. 1. Previously characterized σ -complexes in solution (A) and the solid state (B and C). L_n, ligands; ^tBu, tertbutyl group; Me, methyl.

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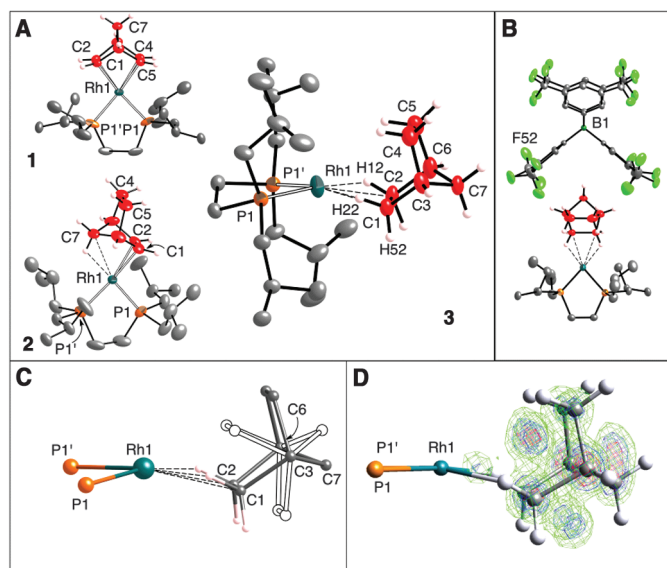
We recognized that the major problem associated with isolating alkane σ -complexes using solution methods is their instability at both the temperatures (above -80°C) and time scales

plex $[\text{Rh}(\text{tBu}_2\text{PCH}_2\text{CH}_2\text{P}^i\text{Bu}_2)(\eta^2\eta^2\text{-C}_7\text{H}_8)][\text{BAR}^{\text{F}}_4]$ **1** (Fig. 2) ^iBu , isobutyl group; Ar^{F} , 3,5-(CF_3) $_2\text{C}_6\text{H}_3$] resulted in an immediate color change from orange to red/purple, which further changed to yellow over time. Following this process using $^{31}\text{P}\{^1\text{H}\}$ solid-state NMR (SSNMR) spectroscopy (fig. S1) (24) revealed the fast consumption of the starting material to form an intermediate species **3**, observed as a broad ABX coupling pattern (coupling constant J for RhP ~ 195 Hz), which then proceeded to give a final stable product **4** as an amorphous solid below 25°C , alongside liberated norbornane [(NBA), observed by solution ^1H NMR spectroscopy]. We have not observed **3** free of **4** by SSNMR. Species **4** was identified by independent synthesis (24) as the zwitterionic complex $\text{Rh}(\text{tBu}_2\text{PCH}_2\text{CH}_2\text{P}^i\text{Bu}_2)(\eta^6\text{-C}_6\text{H}_3(\text{CF}_3)_2)\text{BAR}^{\text{F}}_3$ (25). A $^{13}\text{C}\{^1\text{H}\}$ SSNMR experiment confirmed that hydrogenation of the alkene has occurred in **3**, with the disappearance of diagnostic signals at chemical shift δ 86.6 and 87.4 (26). The lifetime of this intermediate **3** at room temperature varies with the size of the crystalline material: hours for single crystals (0.5 mm^3), minutes for microcrystalline samples. Lower temperatures (-20°C) also effectively halt the transformation of **3** to **4**. Any residual quantity of **1** after hydrogenation remains unchanged over time. Crystallinity is lost in the overall transformation of **1** to **4**, but is retained up to the formation of **3**.

By rapid transfer of a hydrogenated single crystal of **1** (Fig. 3A) to the cryostream (-173°C) of a x-ray diffractometer, a solid-state structure of the intermediate **3** was obtained (27). This procedure is reproducible and was repeated a number of times to obtain the best-quality structure, although this still had $\sim 2\%$ of **1** in the lattice. As the final product **4** is formed in an amorphous phase, it was not observed in the single crystals chosen. A satisfactory structure was also obtained at -60°C . The resulting structural model is consistent with the coordination of an alkane with the metal center (Fig. 3A), $[\text{Rh}(\text{Bu}_2\text{PCH}_2\text{CH}_2\text{P}^i\text{Bu}_2)(\eta^2\text{-}\eta^2\text{-C}_7\text{H}_{12})][\text{BAr}^{\text{F}}_4]$ **3**, in which the alkane (NBA) interacts with a diphosphine-coordinated Rh(I) fragment through two C–H σ -bonds, one each from the C1 and C2 positions in the NBA. The cation in **3** occupies a position on a crystallographic twofold axis, which runs through the middle of the ligand and the metal and relates the two halves of the diphosphine. As a consequence, the NBA ligand is inherently disordered (Fig. 3C). Examination of the Fourier map (Fig. 3D) gives only one chemically sensible structure that fits the electron density, and no restraints were necessary for refinement of the coordinated alkane, although due to the disorder present, C3 and C6 were constrained to enforce the crystallographic symmetry (24). Inspection of the respective C–C distances in the organic fragments in **1** and **3** demonstrates elongation of the C=C double bonds in **1** to C–C single bonds in **3**: C1–C2 expands from 1.363(10) to 1.557(15), whereas C4–C5 expands from 1.363(10) to 1.51(2) Å. However, within a 3σ error, these two C–C distances are not distinguishable. Although the quality of the



Fig. 3. (A) Displacement ellipsoid plots (30% probability) for the cationic components of complexes **1**, **2**, and **3** in the solid state. Selected bond lengths and angles: **1**: C1–C2, 1.363(10) Å; C4–C5, 1.363(10) Å; Rh1–P1, 2.250(4) Å; Rh1–P101, 2.290(4) Å; dihedral C1/C2/P1/P101, –87.8(4)°. **2**: Rh1–P1, 2.2261(15) Å; Rh1–C1, 2.214(13) Å; Rh1–C2, 2.169(12) Å; Rh1–C7 (C–H agostic), 2.576(14) Å; C1–C2, 1.29(2) Å; C4–C5, 1.48(3) Å; P1–Rh1–C7, 161.9(2) Å; dihedral C1/C2/P1/P1, –87.9(10)°. **3**: Rh1–P1, 2.1875(13) Å; Rh1–C1, 2.494(10) Å; Rh1–C2, 2.175.6(8) Å. **(B)** Cationic crystallographically independent cations in sections of the difference electron levels (24). They are due to ~2% of unreacted cations.



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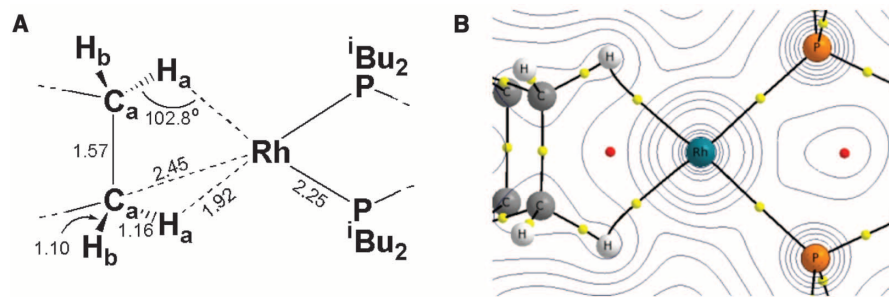


Fig. 4. (A) Key computed distances (in angstroms) and angles (in degrees) for **3** (average values). (B) Contour plot of the total electron density in the {RhH_aH_b} plane. Bond critical points are shown in yellow, ring critical points in red.

data and crystallographically imposed disorder meant that the hydrogen atoms associated with the NBA fragment were placed in calculated positions, the Rh–C1/C2 distances [2.494(10) and 2.480(11) Å, respectively] sit comfortably in the range associated with Rh–C intramolecular agostic σ–C–H interactions (8, 28). They are also similar to Rh–C distances reported for complexes that show intramolecular C–C···Rh σ-interactions (29), although a natural bond orbital (NBO) analysis indicates that such an interaction is not significant in **3** (24). These distances are also consistent with that calculated for the rhodium complex shown in Fig. 1A (2.380 Å) (15). The Rh–P distances in **3** are shorter than in **1** (Fig. 3), consistent with the relative trans influences of alkene and C–H σ-interactions. The hydrogenation of the double bonds in **1** also results in a ~90° twist of the norbornane fragment to best align the C–H bonds with the frontier molecular orbitals of the d⁸–{Rh(L₂)}⁺ fragment (30). In all respects, the structural metrics of **3** point to a square planar Rh(I) center coordinated to a bidentate phosphine and a bidentate alkane—a reassuringly familiar motif in coordination chemistry, albeit with an alkane coordinated [crystallographically characterized chelating σ-bis-silane complexes have been reported (31)]. Dissolving **3**, as formed by the solid-gas reaction, in CDCl₂F gave no evidence for a bound alkane complex in the ¹H NMR spectrum at –110°C (12–15). Instead, approximately one equivalent of free NBA is observed, confirming its formation in the solid state, alongside a species we tentatively describe as a Rh(I) species stabilized by an agostic C–H interaction from an ⁱBu group in the phosphine ligand (32) or a solvent adduct. Methane loss from the rhodium complex in Fig. 1A at –87°C (CDCl₂F solution) has also been suggested to form the corresponding solvent adduct (15). Warming to higher temperatures (–20°C) resulted in the exclusive formation of **4**.

We hypothesized that complex **3** comes from sequential addition of H₂ to the norbornadiene fragment in **1**. To validate this hypothesis, we synthesized the putative monohydrogenated [norbornene (NBE)] intermediate [Rh(ⁱBu₂PCH₂CH₂PⁱBu₂)(η²η²–C₇H₁₀)] [BAR^F₄] **2** by treating the difluorobenzene adduct **6** with NBE in dichloromethane (Fig. 2). The solid-state structure of **2** (Fig. 3A)

shows that the NBE coordinates through one C=C···Rh interaction and one intramolecular C–H···Rh agostic interaction. The NBE fragment adopts an orientation more like that in **1**, with the C=C bond lying perpendicular to the RhP₂ plane. As for **3**, the cation in **2** occupies a position on a crystallographic twofold rotor with the NBE alkene ligand disordered across this axis. Related coordination modes of NBE have been reported (33, 34). The solid-state addition of H₂ to **2**, which is evidently faster than the first hydrogenation (**1** → **2**), yields **3** (within less than 2 min for microcrystalline samples, monitored by SSNMR) and then **4**. The sequence **1** → **2** → **3** demonstrates that the bound organic fragment undergoes a major reorganization in the solid state upon hydrogenation (Fig. 2 and fig. S7) (24), whereas the {RhP₂} fragment remains unchanged. This is confirmed by addition of D₂ to crystalline **1**, which leads to the formation of **4** and exo- and endo-deuteration of the liberated NBA, as has previously been observed in solution for related systems (35). Addition of D₂ to crystalline **2** results in only exo-deuteration. These transformations also occur in solution.

The structures of **2** and **3** have also been investigated computationally. The optimized structure of **3** reproduces the key heavy atom positions well, with an average Rh...C_a distance of 2.45 Å [Fig. 4A; BP86 functional; the computed {P₂Rh(η²η²–C₇H₁₂)} moiety in **3** shows near-C_s symmetry, so average values for all computed data are presented] (24). Short Rh···H_a contacts (1.92 Å) and elongated C_a–H_a bonds (1.16 Å) are also confirmed; the latter is characteristic of a C–H···Rh σ-interaction and is distinctly longer than the C_a–H_b distances (1.10 Å). The presence of two σ-interactions was confirmed by an atoms-in-molecule (AIM) study (Fig. 4B) (36), with curved bond paths between Rh and H_a featuring bond critical points similar to those of an intramolecular agostic interaction (electron density at the bond critical point ρ_{bcp} = 0.059; Laplacian of the electron density at the bond critical point ∇² = 0.200). A corresponding weakening of the C_a–H_a bonds is also seen (ρ_{bcp} = 0.231, compare with 0.275 for C_a–H_b), and a ring critical point confirms the chelating bis-σ-alkane binding mode of the norbornane ligand. An analogous AIM study on **2**

gave similar results (Rh···H_a = 2.00 Å, ρ_{bcp} = 0.045; C_a–H_a = 1.14 Å, ρ_{bcp} = 0.241) while suggesting that the C–H···Rh agostic interaction in that compound is somewhat weaker than the σ-interactions in **3**. Similar trends were computed in a parallel NBO study, which indicated that the major component of the C_a–H_a···Rh interaction in **3** arises from donation of electron density from the C_a–H_a bond to the metal center (24). This is reinforced by π-back donation from Rh-based orbitals into the C_a–H_a σ* orbital, and a second-order perturbation analysis quantifies these interactions at 20.3 and 8.9 kcal/mol, respectively.

We speculate that **3** has a lifetime in the solid state that is sufficient for x-ray crystallographic characterization, because the [BAR^F₄][–] anion provides a well-defined cavity in the lattice that both traps the alkane (Fig. 3B) (24) and accommodates the small structural changes associated with alkene hydrogenation. In this regard, the isolation of **3** is similar to that of the structures shown in Fig. 1, B and C, in which host-guest interactions are suggested to play an important role. In contrast to these structures, however, **3** is closely related to both intramolecular C–H agostic complexes and intermolecular alkane complexes prepared in solution, exploiting the rather open coordination sphere and electron deficiency of the {RhP₂}⁺ fragment. Only upon the major structural change of loss of the NBA and coordination of the arene ring of the [BAR^F₄][–] anion in **4** is crystallinity lost; the respective unit-cell volumes are 5957.62(19) (1), 6008.93(15) (2), 6044.1(3) (3), and 5429.1(3) Å³ (4). This context suggests that the isolation of an alkane σ-complex might be rather system-specific and dependent on both the nature of the alkane (NBA) and the relative packing of cations and anions. Nevertheless, the generation of **3** shows that the rational synthesis of long-lived alkane σ-complexes that can be crystallographically characterized is possible.

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27. Data were collected as described in the supplementary materials, solved with SuperFlip (37), and refined with CRYSTALS (38, 39). Complexes 1, 2, and 3 all occupy a position on a crystallographic twofold axis imposing disorder in the alkane/alkene fragment. See the supplementary materials (24) for full data collection and refinement details.
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Supplementary Materials

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Materials and Methods
Supplementary Text
Figs. S1 to S33
Tables S1 to S7
References (40–70)

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Toward Peace: Foreign Arms and Indigenous Institutions in a Papua New Guinea Society

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In 1990, shotguns and M-16s were adopted into Enga warfare, setting off some 15 years of devastation as youths (~17 to 28) took charge of interclan warfare. In response, people called on elder leaders to adapt customary institutions to restore peace; subsequently, war deaths and the frequency of war declined radically. Data from precolonial warfare, 501 recent wars, and 129 customary court sessions allow us to consider (i) the principles and values behind customary institutions for peace, (ii) their effectiveness, (iii) how they interact with and compare to state institutions of today, and (iv) how such institutions might have shaped our human behavioral repertoire to make life in state societies possible.

In 1990, the Enga of Papua New Guinea (PNG), who number between 400,000 and 500,000 people today, adopted new technologies: shotguns, M-16s, and other semi-automatic rifles to replace bows and arrows in interclan conflicts. Even to the Enga it seemed like the beginning of a never-ending story as two decades of runaway warfare ensued, fueled by a “youth bulge” (1, 2). The age-based power hierarchy of males was overturned; gangs of mercenaries reigned; death tolls soared; trees were razed; gardens uprooted; and houses, and schools, and missions burned to ash. Between 1991 and 2005, Enga fought more than 250 wars (3); organized armed conflicts between political communities, in which the ends were defined by peace agreements (4). From 2006 on, interviews and observations of village court sessions indicated gradual changes in attitudes and practices. Exhausted by war, Enga were calling on local village courts,

sanctioned but not steered by the state, to solve conflicts peacefully through customary institutions. The number of deaths and the duration of wars declined steadily; by 2010 and 2011, few wars were fought in Enga province. Social technology from generations past was adapted to contain the impact of adopted modern technology.

In a recent, important work, Pinker (5) has chronicled the big picture of the decline in violence over human history brought about by developments in our cultural institutions and material milieu that differentially engaged “the demons and angels of our nature.” Violence declines steadily from the anarchy of simple societies as the state achieves a monopoly on the use of force to serve justice. Historians will have their say on the recorded past; however, there is little quantitative evidence on levels of violence for small-scale societies beyond ethnographic snapshots and prehistoric graveyards (6, 7). Certainly, prestate societies have institutions to tame violence (8) ranging from nurturing socialization (9–11) to dispersal (12), duels (13), and compensation (14, 15). Could it be that these did not suffice when small-scale societies evolved into

larger polities, causing a descent into a trough of atrocity during early state formation? After all, our “better angels” (16, 17) must have come about in part through social selection (18, 19), to wit, being penalized or favored by others for partnerships, positions, and privilege via institutional frameworks.

The Enga case provides a rare opportunity to examine the construction and adaptation of institutions to promote peace as well as quantitative measures of their effectiveness. It allows us to address the question of how institutions in small-scale societies counter desires for revenge and tame demons. What are their underlying principles and values? How might they have contributed to shaping our behavioral repertoire to make life in state societies possible? Why are the Enga, who resisted pacification by colonial powers, now eschewing more effective weapons and turning toward peace? Will they be able to do so in the future as they become further integrated into an emerging nation and global economy?

The Enga are highland horticulturalists of PNG who depend on the sweet potato to feed large human and pig populations (20, 21). Men compete passionately in politics, warfare, and the ceremonial distribution of wealth. Women devote themselves to family and subsistence agriculture; when war breaks out, they retreat to their natal clans with their children and pigs. Exogamous clans of 350 to 1000 are the units for most political action including war.

The Enga regarded warfare as a last resort for solving problems; it served to avenge insult or injury, to display strength, and to reestablish balance of power (22). There is little indication that Enga fought largely over land (23). After the sweet potato arrived some 350 years ago (24), there were major population shifts followed by raging wars that altered the social landscape. Subsequently, ceremonial exchange systems called *Tee* arose to reconfigure networks of trade and exchange disrupted by warfare; new religious cults broke cycles of violence (25, 26).

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Around 1850, compensation, formerly paid to allies, was expanded to make peace with the enemy. Clans could and did fight, resolve conflicts, and stay put, reducing migration after warfare (3). Compensation cost precious pigs and valuables, providing a strong incentive to establish rules to contain warfare. When well-organized by skilled leaders, warfare and peace-making had economic motivations: to reestablish ties, balance of power, and respect between clans so that wealth for the many forms of ceremonial exchange called *Tee* could continue to flow freely (25, 26). Warfare and exchange thus became entwined.

The Australian Colonial Administration's pacification of PNG societies (15, 27) reached Enga in the 1950s and 1960s, making way for missions and new economic opportunities. Many traditions waned, including bachelors' cults, ancestral cults, and separate men's and women's houses. The last great *Tee* cycle of enchain exchange was held in 1978 to 1979 (25), ending one close link between warfare and exchange. Guns in the hands of the colonial administration brought peace; Enga turned to "fighting in court" (28). A few years before independence, warfare resurged as social inequalities grew. The western justice system had failed to restore relations by mediation and material compensation and ignored local politics and future relationships (28).

In 1974, the Village Court Act came into effect to serve parochial judicial needs by harnessing existing local potential (22). Village courts were to apply "custom" to achieve substantial justice. Custom was imprecisely defined and changeable; community harmony was prioritized (29). Respected local leaders elected as magistrates found themselves part of the national legal system with little training beyond instruction about the limits of their jurisdiction (29). State support was limited to minimum wages, clerks to keep records, provision of uniforms, a provincial office, and sporadic police support. In 1982, a branch of the village courts, Operation Mekim Save (OMS), was established to settle tribal wars; initially OMS favored judicial arbitration by village court magistrates backed by punitive police power (28).

Enga see village courts as their own institutions because they choose their own magistrates and because decisions are not steered, supervised, or enforced by the state. Village courts often dodge the limits of jurisdiction by hearing serious crimes and mediating until the opposing parties reach a settlement. Enga communities have taken the initiative to create a lower echelon of courts that fall outside of the national justice system: *wari* (worry) courts. One or two community leaders mediate disputes on the spot in *wari* courts (22); if mediation fails, complaints are taken to village courts.

Throughout the 1980s, OMS left much to be desired with slow response and often-brutal police raids favoring one side (29, 30). Discontent festered in a weak state (31). In 1990, guns, formerly rejected in warfare to avoid carnage (21), were adopted by some hot-headed youths to the

dismay of the majority, igniting an arms race (22). High-powered rifles were obtained from businessmen and politicians or "acquired" via the police and army (32). The key triggering incidents of recent wars remained similar to those of the past: murder, revenge, theft, and land disputes (table S1).

Guns altered the leadership, tactics, and outcomes of war. Ambushes and raids, executed outside the control of elder clan leaders and OMS replaced pitched battles. Warring clans employed mercenaries skilled in the use of semiautomatic weapons, called "Rambos" or "hiremen," who fought largely for brotherhood and fame and to "carry out justice where government had failed" (supplementary text). In the late 1990s, warriors formed "teams" of peers led by Rambos that cross-cut clan boundaries (3), similar to some inner-city gangs (33). Men who had never cooperated before were bonded through oaths of loyalty, rituals of confession, traditional fight magic, and victory celebrations. Over time, teams colonized the problems of surrounding clans to fight out their own vendettas, accelerating warfare (3). The last vestige of state control over warfare, police support for preventative orders, was withdrawn in most cases because police were no match for warriors with similar arms and superior experience, knowledge of terrain, language, and people (34).

As guns spread, the number of wars in Enga increased rapidly (Table 1); the number of deaths per war rose from a mean of 3.7 (21) in precolonial times to 17.8 between 1991 and 2000. Wars can last from weeks to several years. When both sides are exhausted, elder leaders with economic means and political skills take over peace

negotiations. Unable to hold and use land vacated, winners request a moderate payment from the losers to recognize defeat and let them resettle. Formerly, wars reestablished balance of power (23, 30) so that exchange could flow; recent wars leave imbalance, widespread destruction, and thousands of refugees (3). Refugees are adequately provided for by hosting clans but nonetheless incur deep emotional scars. Public servants fear venturing into rural areas to initiate development projects; in cities, men from enemy clans are targets for payback murders.

Starting around 2005, Enga began to resist entrapment in runaway violence. Attitudes changed. This did not happen over night, for there were two opposing forces at work. Rambos and teams aggressively sought to colonize the problems of others taking advantage of the frenzy around the 2007 elections and the 2009 tension-fraught bi-election in district A. The number of wars increased from 94 in 2004 to 2006 to 149 in 2007 to 2009 (Table 2). Simultaneously war-weary citizens began to withdraw support for warriors and to turn to OMS to put an end to armed conflicts soon after they broke out. Consequently number of deaths before wars were ended decreased (Table 1): from 1991 to 1995, 23% of wars were ended after one to five deaths, whereas from 2006 to 2010 74% of wars were ended after one to five deaths. By 2010 and 2011, citizens prevailed, and war was widely rejected as a solution to solve problems (Table 2).

Why the turn toward peace? Three factors prevailed: exhaustion and economic hardship, church influence, and an effective OMS. Tradi-

Table 1. Number of wars, deaths per war, and deaths before war ended by five year interval from 1991 to 2010.

Deaths before war ended	1991–1995		1996–2000		2001–2005		2006–2010	
	<i>n</i> wars	% wars	<i>n</i> wars	% wars	<i>n</i> wars	% wars	<i>n</i> wars	% wars
1–5	10	23%	34	40%	83	58%	168	74%
6–10	17	39%	24	28%	25	17%	32	14%
11–50	13	29%	20	23%	33	23%	25	11%
51–300	4	9%	8	9%	3	2%	1	1%
Total	44	100%	86	100%	144	100%	226	100%
Total number of deaths	836		1475		1363		1142	
Average number of deaths per war	19		17		10		5	

Table 2. Number of wars and mean number of deaths per war from 2001 to 2011. The Porgera district has been omitted. District C had no wars in 2011.

	2001–2003		2004–2006		2007–2009		2010–2011	
	Total wars	Average no. deaths	Total wars	Average no. deaths	Total wars	Average no. deaths	Total wars	Average no. deaths
District A	13	13	27	5	59	5	3	6.7
District B	31	6	17	6	26	7.3	7	6.3
District C	18	10	19	6	18	3.6	13	5
District D	21	26	17	7	39	2.8	2	2
District E	17	7	14	2	7	2	3	3
Total	100	12	94	5	149	4.5	28	5.1

tionally, Enga fought to restore balance so that exchange could flow. Most Enga who have experienced recent wars have incurred unprecedented losses with no gains for exchange. Exhaustion and imbalance prevail. Even youths from the initial cohort of modern warriors have matured into family men with more assets to lose. Lastly, the Enga provincial government has prioritized education, giving youths new visions of prosperity for the future. Without social and material rewards, the enthusiasm of warriors wanes (supplementary text).

Church influence in promoting peace has been strong since the 1950s, with a phase of proactive peace initiatives in the 1980s and early 1990s (35, 36) that floundered as warfare with modern weapons intensified. Churches provide a legitimate institution, ideology, and fellowship for those who eschew warfare, even though the Enga norm, “Do unto others as they do unto you,” persists together with new norms such as “Money is life.” The charisma of locally led religious celebrations, with their colorful regalia, music, and festivities, disrupts cycles of vengeance and prepares the emotional landscape for peace. Nonetheless, no Rambo interviewed felt that Christianity had convinced him to stop fighting nor would do so if need arose. Women’s peace movements have not been as prominent as elsewhere in the PNG highlands (37, 38).

Lastly, OMS is key for peace-making because as third parties they have the ability and authority to mediate emotional, social, and material solutions to intergroup conflicts. Armed with cell phones and better transport to reach trouble spots in time, OMS has become more effective in the past years. In a sense, OMS gained when police backing dwindled; people had to turn to OMS to contain wars knowing that no outside force would intervene. OMS magistrates sidestep their limits of jurisdiction by mediating compensation for serious crimes. Legal authorities do not object because the state is not autonomous from local social forces (29, 39); if peace can be achieved, development will succeed, and that success, in turn, will lead to a more stable peace.

What makes OMS so effective? Legitimization by the state gives authority; lack of supervision allows OMS magistrates to work under few constraints. OMS magistrates say that they “speak the language, know the hearts of people, and do not just read from some law book.”

Magistrates weather the rain, the sun, and the occasional raucous drunk or pig while people air complaints in public and the crowd responds. They organize evidence into a settlement that is sensitive to current conditions, emotions, and values. OMS magistrates receive the privileges of high standing in the community for their hard work, including protection from violence.

Table 3 compares outcomes for serious criminal cases that were brought to the district court with those heard by the OMS village court in the provincial capitol of Wabag (22). Ninety-eight percent of OMS cases reached or approached resolution via compensation as a means of restorative justice. Only 10% of district court cases concluded with a jail sentence; the rest were struck out when witnesses did not appear or the case was withdrawn by the plaintiff and taken to OMS. Jail sentences do not satisfy as material reparations do.

What are the principles that quell emotions and resolve serious disputes? For 45 serious cases, magistrates gave one or more of the following justifications (22): 16 cases, lack of respect for the emotions of the complainant; 13 cases, breach of respect for property; 5 cases, untruths; 13 cases, failing to meet traditional obligations; and 12 cases, failure to exercise restraint under rage or intoxication. These norms and values are expressed in precontact maxims that guide village court decisions today (supplementary text). Kinship ties were called on to settle matters peacefully in 33% of the cases. In 26% of the cases, parties were encouraged to go home, “drink Coca-Cola,” and first try to resolve the dispute by themselves (40). Christian values or human rights principles were not explicitly cited to justify decisions; magistrates did cite women’s rights, a prominent theme in the current media.

In paying compensation, the offender accepts liability, acknowledges the pain of loss, and compensates materially. Kin outside the clan assist donor clans in assembling wealth; politicians and businessmen donate large sums to increase their popularity. When done well, compensation changes hearts so that people let go and proceed with productive relations; when poorly done, hostilities resume. The cost of paying compensation is mitigated by anticipated social and political gains for those who honor financial responsibilities, show respect, and refrain from

violent retribution. Those who cost the clan repeated compensation or fail to contribute are marginalized as “rubbish men” (supplementary text). Peace-making provides the framework for social selection of behavioral predispositions that are the building blocks for the modern state (41).

This short exposition excludes many important aspects of war and peace-making to be explored in future publications to provide deeper understanding. These include the role of history, cultural context, the state, religion, leadership, oratory, war magic, and above all, insights that can be gained from comparison with other PNG societies (15). Nonetheless, it is possible to propose some answers to the opening questions. Local Enga institutions were indeed effective in containing violence and restoring order in two tumultuous periods. The first was precolonial, when peace-making was devised to allow clans to fight, pay reparations, stay put, and renew ties of co-operation. The reduction of subsequent postwar migrations attests to its effectiveness (3, 25). The second was postcolonial, when the state’s sanctioning of indigenous peace-making together with the influences of Christianity yielded similar success. Death rates in war per 100,000 per annum were 81 in 1990 when guns were adopted, 91 in 2000 when wars with modern weapons raged, and 19 in 2011 when Enga sought peace (22). The inclusion of nonwar homicides per year from 2006 to 2011 from OMS records yields 103 deaths per 100,000 for 1990, 110 for 2000, and 32 for 2011. These figures are lower than Pinker’s (5) estimate of an average of 524 deaths per 100,000 and Keeley’s (6) of 500, underscoring the variation in nature and effectiveness of peace institutions in tribal societies.

New institutions build on former rules, norms, and values; history matters (42, 43). The Enga are fortunate to have the legacy of compensation to reduce violence that could be integrated into the legal system of a democracy (15). However, Enga compensation is based on relationships of a small-scale society and has drawbacks as scale increases. In a world of drugs, alcohol, cell phone affairs, and high mobility, responsible citizens must cover for the crimes of the irresponsible. Compensation is expensive: an average of 26 pigs and K1239 (~U.S. \$500) per death since 1991 (3). Formerly, clan members contributed to repair the wrongs of “brothers,” moved by life-long co-residence, emotional bonds, and the need for a strong, protective corporate group. In a society of growing scale and mobility, clan members are often brothers by name alone. Strapped by monetary needs for education and modern amenities, responsibility is narrowing to extended family members and politicians seeking to gain popularity through contributions. Many Enga question if and how compensation will continue to be effective in the future outside of internal village disputes or major threats.

Recent trends suggest that interclan warfare as a response to insult or injury may soon be a practice of the past; people have experienced

Table 3. Outcomes of cases heard in district and village courts in 2011. Cases for both courts include a similar proportion of charges concerning murder, rape, assault and property (22).

Decision	District court		OMS village court	
	<i>n</i>	%	<i>n</i>	%
Fine or jail sentence	4	10%	1	2%
Struck out	18	43%	0	0%
Withdrawn	20	47%	0	0%
Compensation order	0	0%	17	40%
Compensation settlement mediated	0	0%	9	29%
Mediated agreement reached to settle out of court	0	0%	12	29%
Total	42	100%	39	100%

modern war and recognized its futility. In the face of rapid change, the future is hard to predict. If the large sums of income from natural resources and foreign aid are applied to development during this time of relative peace, the people of Enga will have more to lose and may continue to turn away from war. However, there is a burgeoning population of discontented youths, and politics are heating up as multinationals invest billions to extract the resources of an otherwise poorly developed PNG. Perks for those in power are many. In some areas, gangs are already serving the interests of politicians (30); a new round of warfare could erupt over the politics of tangible resources. If this happens, local institutions founded on principles of kinship, respect, and restorative justice will not suffice, and the Enga may find themselves in another cycle of violence as the scale of their society increases. This was true for many societies in the past (5, 44) and is still the case for societies in similar transitions today.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6102/1651/DC1
Materials and Methods
Supplementary Text
Table S1
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Database S1

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Adaptive Sleep Loss in Polygynous Pectoral Sandpipers

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The functions of sleep remain elusive. Extensive evidence suggests that sleep performs restorative processes that sustain waking brain performance. An alternative view proposes that sleep simply enforces adaptive inactivity to conserve energy when activity is unproductive. Under this hypothesis, animals may evolve the ability to dispense with sleep when ecological demands favor wakefulness. Here, we show that male pectoral sandpipers (*Calidris melanotos*), a polygynous Arctic breeding shorebird, are able to maintain high neurobehavioral performance despite greatly reducing their time spent sleeping during a 3-week period of intense male-male competition for access to fertile females. Males that slept the least sired the most offspring. Our results challenge the view that decreased performance is an inescapable outcome of sleep loss.

Sleep is a prominent yet enigmatic part of animal life (1). In humans, and other mammals, sleep restriction and fragmentation

diminish waking neurobehavioral performance (such as attention, motivation, sensory-motor processing, and memory), often with adverse con-

sequences for the individual and society (2–5). Sleep loss even impairs the performance of innate behavioral displays (6). These findings suggest that sleep performs essential restorative processes that sustain adaptive brain performance (1). An alternative view posits that sleep may be simply a state of adaptive inactivity that conserves energy when activity is not beneficial (7). This adaptive inactivity hypothesis proposes that the marked

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Fig. 1. Behavioral and morphological traits of polygynous male pectoral sandpipers contributing to reproductive success. (A) Male flight display characterized by throat inflation and hooting sounds. (B) Male (left) engaged in precopulation ground display to a female; illustration of sexual size dimorphism. (C) Males engaged in territorial ground display. (D) Males engaged in physical fight. (E) Male standing vigilant. (F) Males engaged in aerial chase. [CREDIT: (A) to (D) and (F) Wolfgang Forstmeier, Max Planck Institute for Ornithology; (E) B.K.]

variability in sleep duration observed across the animal kingdom reflects varying ecological demands for wakefulness, rather than different restorative requirements. According to this hypothesis, animals can evolve the ability to dispense with sleep when ecological demands favor wakefulness.

Sexual selection has led to the evolution of costly morphological, physiological, and behavioral traits (8). In polygynous species, in which postzygotic paternal investment in young is absent, male reproductive fitness is determined exclusively by access to fertile females. For a polygynous male to maximize his fitness, he must successfully engage in competitive displays and physical fights with other males and in courtship displays with females. Although the time available for engaging in visual displays is limited by day length at lower latitudes, it is seemingly unlimited for species that breed in the high Arctic, where the Sun never sets during the mating period. However, under these conditions the need for sleep might limit the time available for pursuing and displaying to fertile females. As such, strong sexual selection may favor an ability in males to forgo sleep without experiencing diminished neurobehavioral performance (9, 10). Such

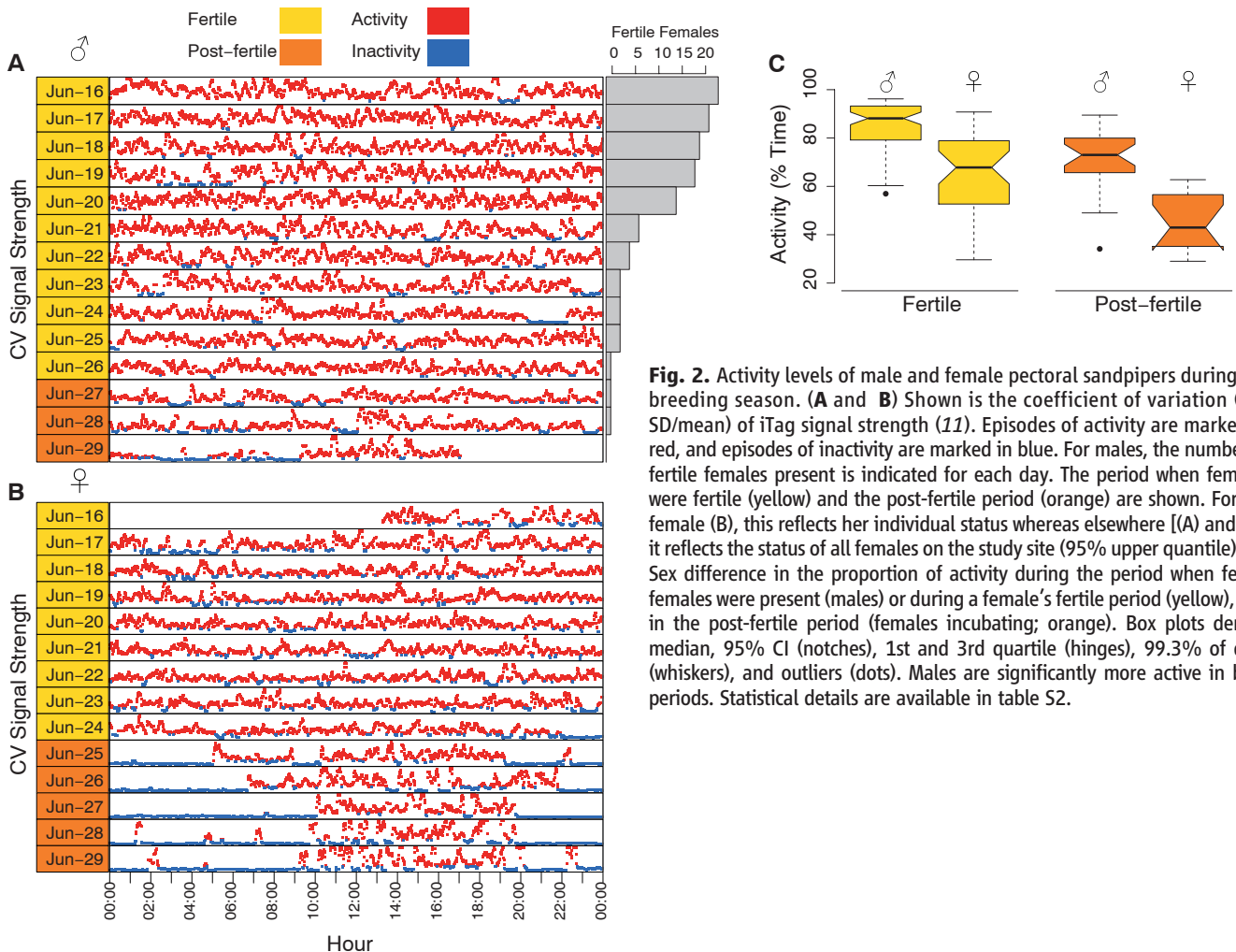


Fig. 2. Activity levels of male and female pectoral sandpipers during the breeding season. (A and B) Shown is the coefficient of variation (CV; SD/mean) of iTag signal strength (11). Episodes of activity are marked in red, and episodes of inactivity are marked in blue. For males, the number of fertile females present is indicated for each day. The period when females were fertile (yellow) and the post-fertile period (orange) are shown. For the female (B), this reflects her individual status whereas elsewhere [(A) and (C)] it reflects the status of all females on the study site (95% upper quantile). (C) Sex difference in the proportion of activity during the period when fertile females were present (males) or during a female's fertile period (yellow), and in the post-fertile period (females incubating; orange). Box plots denote median, 95% CI (notches), 1st and 3rd quartile (hinges), 99.3% of data (whiskers), and outliers (dots). Males are significantly more active in both periods. Statistical details are available in table S2.

flexibility in sleep needs could challenge the view that a fixed amount of daily sleep is needed to maintain performance.

We examined the relationship between time spent awake and reproductive output in male pectoral sandpipers (*Calidris melanotos*). The species is characterized by strong sexual size dimorphism (Fig. 1B and table S1) and a polygynous mating system without pair bonds. Males spend large amounts of time defending their territory against intruders and displaying to or chasing females. Male territories vary in size depending on density (fig. S1). Breeding males engage in display flights over females (Fig. 1A and audio S1) and in ground displays, a behavior that precedes copulation (Fig. 1B and movie S1). Females are very reluctant to copulate (11). Males often engage in territorial interactions, including parallel walks and physical fights with other males (Fig. 1, C and D). Males remain vigilant for intruders and females (Fig. 1E) and engage in aerial chases of females, often in direct competition (Fig. 1F). Males and females associate only temporarily for courtship and copulation; incubation and chick rearing are done exclusively by the female (12).

We studied a population of pectoral sandpipers on the Arctic tundra when females were

fertile and post-fertile (incubating). We recorded the activity pattern of virtually every resident male pectoral sandpiper and a representative sample of females using a radiotelemetry-based system developed for this study (11). The system also recorded interactions between males and females. These data showed that males were more active than were females during both the fertile and post-fertile period ($n = 149$ individuals, $P < 0.001$) (Fig. 2, A to C, and table S2). Male activity declined once fertile females were no longer available ($P < 0.001$) and approached the level of female activity during the fertile period (Fig. 2C and table S2). The overall level of activity varied considerably across males, even when fertile females were available (Fig. 2C). In the most extreme case, a male was active >95% of the time for a period lasting 19 days.

Using a recently developed datalogger (13), we obtained combined electroencephalogram (EEG) and neck electromyogram (EMG) recordings from males on their territory (11). These recordings allowed us to determine whether inactive males actually spent more time sleeping than did active males or simply spent more time sitting quietly while awake. Sleep typically occurred in brief bouts between periods of activity (Fig. 3A). As in other birds (14, 15), wakefulness

and sleep were associated with high and low EMG activity, respectively (Welch t test, $t_{13,9} = 14.4$, $P < 0.001$) (Fig. 3B). Indeed, the sandpipers rapidly transitioned from active wakefulness to sleep without engaging in quiet wakefulness (Fig. 3A). Consequently, inactivity and activity are valid proxies for sleep and wakefulness in this context, respectively. As suggested from the activity recordings, time spent sleeping varied considerably across males (Fig. 3C), with the shortest sleeping 2.4 hours and the longest 7.7 hours (5.2 ± 0.5 , mean $\pm$ SEM, $n = 11$ males). The total time males spent sleeping was correlated with the number of sleep episodes [correlation coefficient (r) = 0.79, $t_9 = 3.9$, $P = 0.004$], mean duration of individual sleep episodes ($r = 0.59$, $t_9 = 2.2$, $P = 0.057$), and maximum sleep bout duration ($r = 0.66$, $t_9 = 2.6$, $P = 0.027$) (Fig. 3D). We also examined the EEG for signs of deeper (more intense) sleep in short-sleeping males. As in mammals, avian non-rapid eye movement (REM) sleep-related EEG slow wave activity (SWA) increases as a function of the duration and intensity of prior wakefulness (16) and therefore may reflect sleep need and intensity. Despite experiencing more fragmented sleep, the males that slept the least showed the greatest SWA ($r = -0.60$, $t_9 = -2.27$, $P = 0.049$) (Fig. 3E), suggesting that they

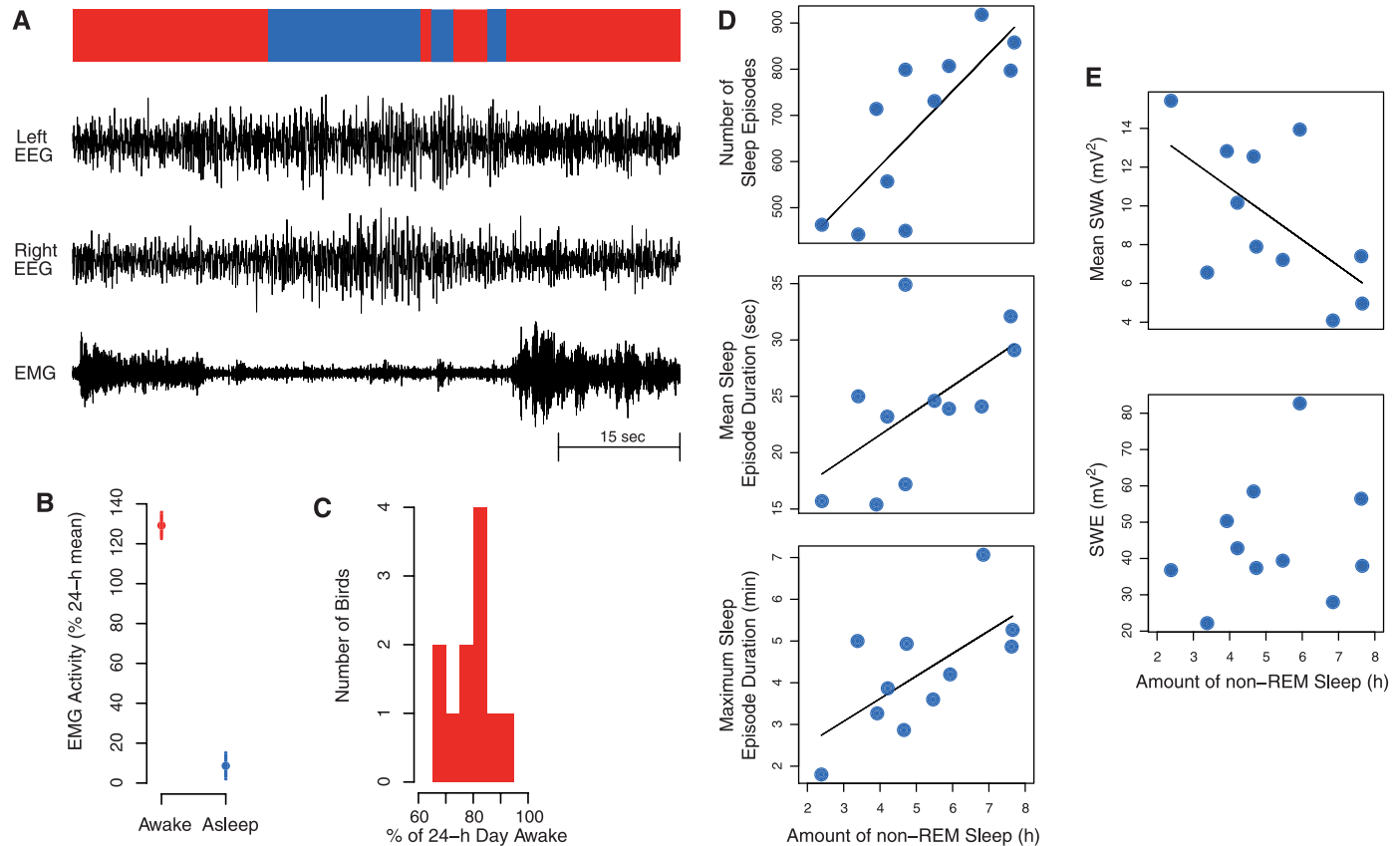


Fig. 3. Short, fragmented, but deeper sleep in extremely active males. (A) EEG and EMG signs of wakefulness (red) and non-REM sleep (blue) in a pectoral sandpiper. (B) EMG activity (mean $\pm$ 95% CI) was highest during wakefulness, reflecting virtually continuous movement. (C) Large variation in the amount of

EEG/EMG-defined wakefulness across birds. (D) Short-sleeping males had fewer and shorter sleep episodes relative to long-sleepers. (E) SWA during non-REM sleep was greater in short-sleeping males, but SWE was not, suggesting that short-sleeping males experienced a deficit in sleep despite sleeping more deeply.

compensated, at least partially, for sleep loss by sleeping deeper. To determine whether the increase in sleep intensity compensated fully for the lost sleep, we next calculated slow wave energy (SWE; mean SWA $\times$ number of non-REM sleep epochs), a measure of cumulative non-REM sleep that accurately tracks sleep need during chronic sleep restriction (17). Short-sleeping males should show greater SWE than that of long-sleeping males if they compensated completely for sleep loss by sleeping deeper; however, we did not find an inverse correlation between sleep duration and SWE ($r = 0.22$, $t_9 = 0.70$, $P = 0.50$). Consequently, short-sleeping males still experienced a deficit in sleep.

We examined the relationship between male activity during the fertile period and the number of male-female interactions (telemetry-based) and resulting paternity (using microsatellite markers) (11). The number of different females with which a male interacted (Fig. 4A) and the total number of interactions with females (Fig. 4B) were predicted by the amount of time males were active ($n = 73$ males, $P < 0.01$ and $P = 0.017$, respectively) (table S3). Moreover, time spent active was significantly correlated with the number of females with which a male sired young ($n = 73$

males, $P = 0.004$) (Fig. 4C) and with the total number of young he sired ($P = 0.004$) (Fig. 4D). Indeed, the males that sired the most young were among the most active.

To determine whether there are long-term costs associated with sleep restriction and fragmentation during the breeding season, we examined the return rate and reproductive success of males across years. Males rarely returned to the study site ($n = 13$ out of 640 males across 6 years). Nonetheless, the probability of return was 10% higher [confidence interval (CI) 95%: 0 to 20%] for successful males than for males that did not sire offspring (generalized linear model with binomial error, two-tailed test, $P = 0.08$), a trend opposite to that expected if short-sleeping males experienced reduced survivorship. Moreover, 58% of the returning males were successful in siring at least one offspring in the second year, compared with only 20% of other males in the population (Fisher's exact test, $P = 0.005$). This suggests that reproductively successful males either survive better across years than do unsuccessful males or show greater fidelity to areas where they have successfully reproduced.

Reduced sleep has been described in caged white-crowned sparrows (*Zonotrichia leucophrys*

gambelii) and Swainson's thrush (*Catharus ustulatus*) exhibiting nocturnal migratory restlessness (14, 18). However, in contrast to pectoral sandpipers, which only compensated partially for sleep loss by sleeping more deeply, songbirds compensated for sleep loss at night by increasing the time spent drowsy and sleeping during each day. Furthermore, sleep loss was associated with decreased performance on certain cognitive tasks (19), and the relationship between sleep duration and reproductive output was not addressed. Similarly, in dolphins the relationship between extended periods of constant swimming with environmental awareness and reproductive success has not been determined (20, 21).

Three pieces of evidence suggest that the amount of wakefulness is under strong sexual selection. First, males were more active than were females when females were fertile. Second, the total time a male was active during the fertile period was a strong predictor of his reproductive output. Third, the relationship between activity and reproductive output is probably directly related to competition for access to fertile females because a male's reproductive success was strongly related to the proportion of time he was observed showing territorial or courtship behavior (table S4). Moreover, male activity was also a good predictor of the total number of interactions with females, and of the total number of different females with which a male interacted.

The recent discovery of sleep-like neuronal activity occurring locally in the cortex during wakefulness in sleep-deprived rats (5) raises the possibility that the "missing" sleep occurred in a similar manner in short-sleeping male pectoral sandpipers. This is unlikely, however, because local sleep only occurred while the rats were immobile, and performance on a foraging task was impaired if local sleep occurred in the motor cortex shortly before the task. The high reproductive success of our short-sleeping males suggests that they were not similarly impaired.

Male pectoral sandpipers forgo sleep to ensure paternity, exactly as the adaptive inactivity hypothesis predicts. However, if sleep is expendable, why do some males sleep more than others when fertile females are available? Although defeated males may give up and resort to sleeping to save energy, the energy saved by sleeping instead of sitting quietly awake (22) would need to offset the potential cost of increased predation risk during sleep (23). Moreover, the increase in sleep intensity in short-sleeping males suggests that sleep serves a restorative function. In this case, long-sleeping males may lack genetic traits that enable short-sleeping males to maintain high performance on little sleep. Indeed, interindividual variation in neurobehavioral vulnerability to sleep loss was recently linked to genetic polymorphisms in humans (24). If there is a genetic basis to male-male variability in sleep duration and resulting neurobehavioral performance in pectoral sandpipers, then the persistence of the long-sleeping phenotype suggests that it may be equally suc-

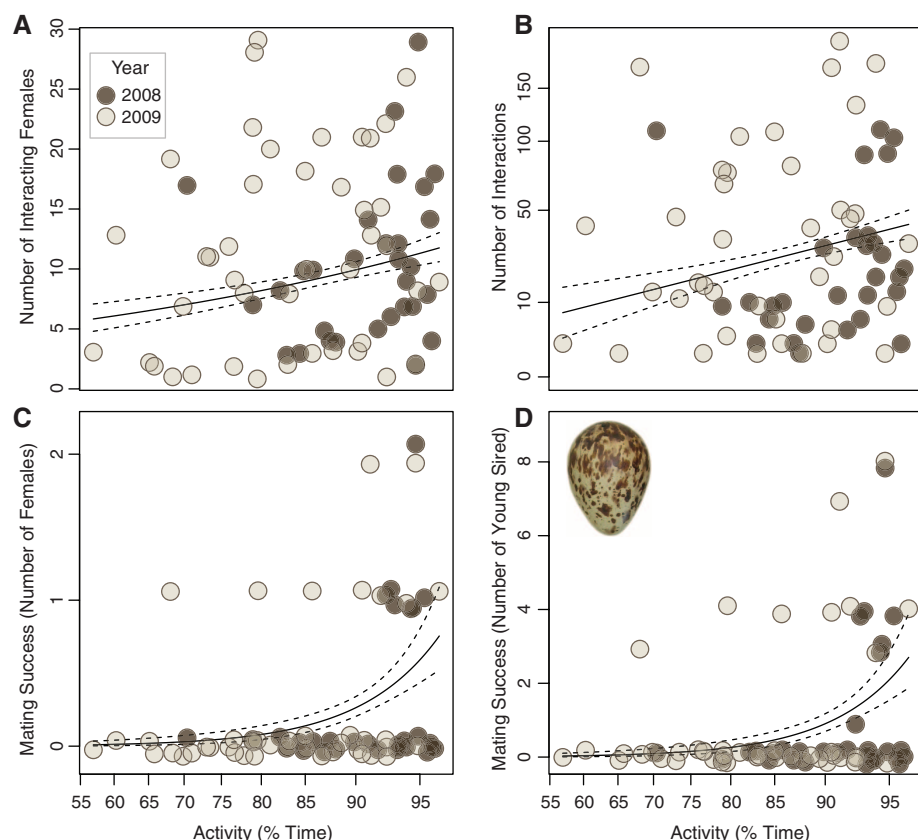


Fig. 4. High activity contributes to male reproductive success. The proportion of time a male was active during the period when fertile females were present is a significant predictor of (A) the total number of different females with whom he interacted, (B) the total number of interaction bouts with females, (C) the number of females with whom he sired offspring, and (D) the total number of offspring he sired in a given year. Shown are the raw data and the fitted lines together with 95% CIs. Statistical details are available in table S3.

cessful over the long term. However, our limited across-season data suggests that short-sleeping males may actually perform better than do long-sleeping males over the long term, suggesting ongoing sexual selection instead. Ultimately, a greater understanding of potential short- and long-term costs of reproductive sleep loss in pectoral sandpipers may provide insight into the evolution of this extreme behavior, as well as the ongoing debate over the functions of sleep (25) and its relationship to health and longevity in humans (26, 27).

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Supplementary Materials

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Mutations in the *neverland* Gene Turned *Drosophila pachea* into an Obligate Specialist Species

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Most living species exploit a limited range of resources. However, little is known about how tight associations build up during evolution between such specialist species and the hosts they use. We examined the dependence of *Drosophila pachea* on its single host, the senita cactus. Several amino acid changes in the Neverland oxygenase rendered *D. pachea* unable to transform cholesterol into 7-dehydrocholesterol (the first reaction in the steroid hormone biosynthetic pathway in insects) and thus made *D. pachea* dependent on the uncommon sterols of its host plant. The *neverland* mutations increase survival on the cactus's unusual sterols and are in a genomic region that faced recent positive selection. This study illustrates how relatively few genetic changes in a single gene may restrict the ecological niche of a species.

Losses of enzymatic activities are frequent during evolution (1). For example, humans lost the ability to produce nine amino acids and six vitamins, for which we rely on our diet (2). The reasons for such losses are unknown, but it is generally believed that “superfluous” metabolic activities were lost by chance during evolution (3). We examined the dependence of the fly *Drosophila pachea* on the senita cactus (*Lophocereus schottii*), a plant species endemic to the Sonoran desert (northwestern Mexico and southwestern United States). In insects, developmental transitions and egg production are regulated by the steroid hormone ecdysone (4).

However, *D. pachea* has lost the first metabolic reaction in the ecdysone biosynthetic pathway, i.e., the ability to convert cholesterol into 7-dehydrocholesterol (7DHC) (Fig. 1A) (4–7). The senita cactus, which *D. pachea* requires as a host (5), does not contain common sterols and is the only plant in the Sonoran desert (7) known to produce Δ^7 -sterols such as lathosterol (6). *D. pachea* flies do not reach the adult stage if not raised on senita cactus, but supplementing standard food with senita cactus or with 7DHC fully restores *D. pachea* viability and fertility (5), indicating that Δ^7 -sterols are essential compounds required for *D. pachea* development and survival.

Interestingly, *D. pachea* appears to depend on the senita cactus solely for its sterols, as we raised *D. pachea* on an artificial diet supplemented with 7DHC for more than 4 years (~60 generations) with no apparent defect (8).

Conversion of cholesterol into 7DHC is catalyzed by the evolutionarily conserved Rieske-domain oxygenase Neverland (NVD) in insects and nematodes (9, 10). To investigate whether mutation(s) in *nvd* are responsible for *D. pachea* dependence on its host cactus, we sequenced the *nvd* coding region (8) from *D. pachea* and the three most closely related species—*D. nanoptera*, *D. acanthoptera*, and *D. wassermani*—which feed on other cacti (11) (tables S1 and S2 and fig. S1). No stop codon or insertions/deletions were found in the *D. pachea* sequence, but the ratio of rates of nonsynonymous substitution (d_N) over synonymous substitution (d_S) is significantly higher in the branch leading to *D. pachea* (table S3 and fig. S2). We noticed that several amino acids showing high conservation across insects and vertebrates are different in *D. pachea* NVD (Fig. 1, B and C).

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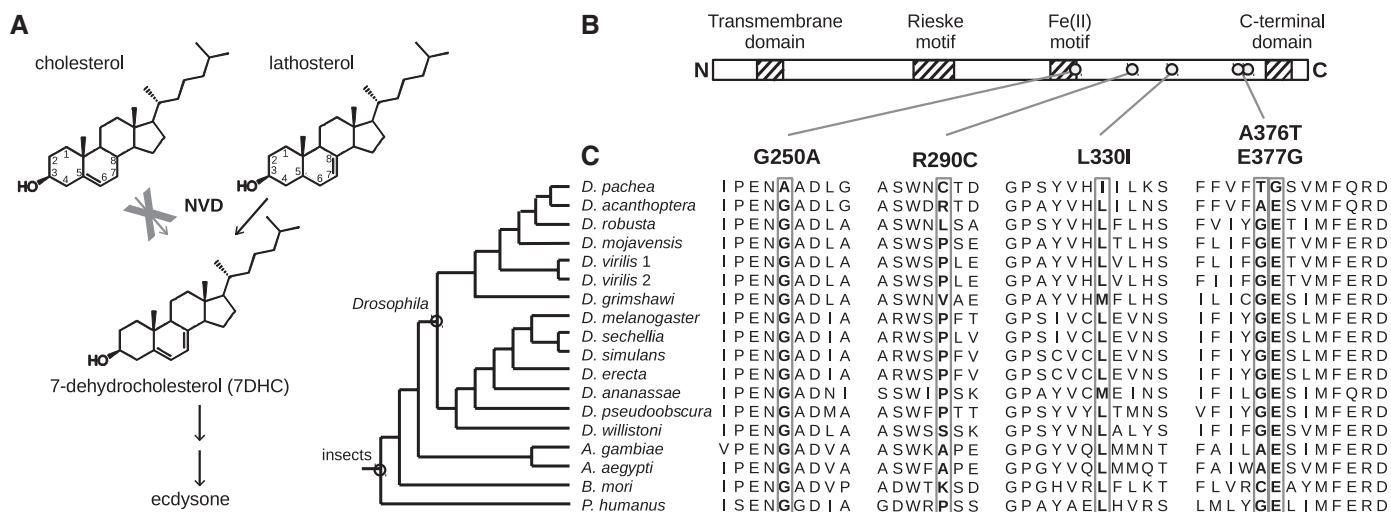


Fig. 1. Presumed ecdysone biosynthetic pathway (A) and NVD in *D. pachea*. (B) NVD protein structure. (C) Alignment of multiple NVD protein sequences. Five mutations (boxes) were tested in vitro. For an alignment of full NVD protein sequences with additional insect species, see fig. S8.

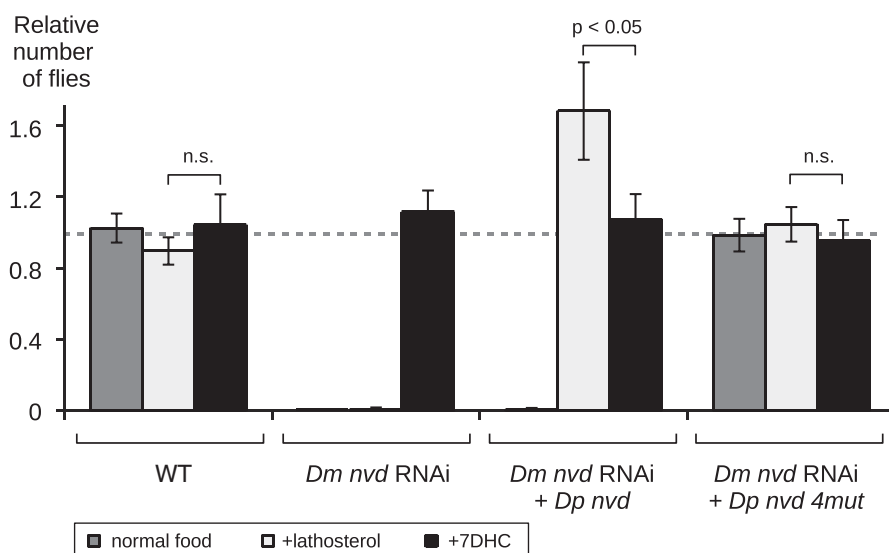


Fig. 2. Fly survival on various food media. WT, control flies; *Dm nvd* RNAi, RNAi knockout of WT *D. melanogaster nvd*; +*Dp nvd*, rescued with WT *D. pachea nvd*; +*Dp nvd* 4mut, rescued with *D. pachea nvd* A250G I330L T376A G377E. The proportion of flies of each genotype is indicated relative to the number of *UAS-nvd* RNAi *Sb* male siblings (8) (table S4). Bars show average, and error bars are mean $\pm$ SE.

We observed that in *D. pachea* third instar larvae, as in *D. melanogaster* (9) and *D. acanthoptera*, *nvd* is only expressed in the prothoracic gland (fig. S3), an organ whose sole known function is ecdysone production (12). Therefore, we conclude that NVD function, if any, should be related to steroid hormone production.

The senita cactus does not contain cholesterol or 7DHC but does produce three other sterols—lathosterol, campestenol, and schottenol (6)—that, if used as precursors for steroid hormone synthesis, are expected to lead to different steroid hormones—respectively, 20-hydroxyecdysone, makisterone A, and makisterone C (fig. S4)—due to the inability of *Drosophila* to dealkylate phytosterols (13). Steroids from *D. pachea* ex-

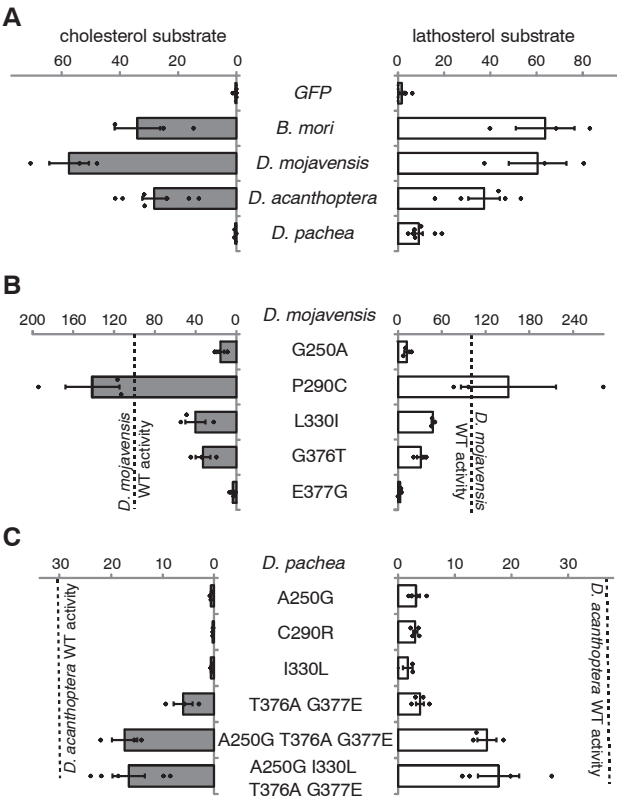
tracts were separated by high-performance liquid chromatography, and fractions of interest were analyzed with mass spectroscopy. We detected ecdysone and 20-hydroxyecdysone but no trace of makisterone A or makisterone C (fig. S5). These results suggest that *D. pachea* only uses lathosterol and not the other senita cactus sterols as steroid hormone precursors.

Because conversion of cholesterol into 7DHC biochemically resembles the transformation of lathosterol into 7DHC (Fig. 1A), we hypothesized that *D. pachea* NVD converts lathosterol rather than cholesterol into 7DHC (14). To test this hypothesis, we generated transgenic *D. melanogaster* flies in which the endogenous *nvd* gene is shut down by RNA interference (RNAi)

and replaced by *D. pachea nvd*. The *D. melanogaster nvd* RNAi flies do not develop on regular fly food (9) or on food supplemented with lathosterol, yet they reach the adult stage on regular fly food supplemented with 7DHC (9) (Fig. 2 and table S4). As expected, introduction of *D. pachea nvd* into *D. melanogaster nvd* RNAi flies rescues development on food supplemented with lathosterol but not with cholesterol (Fig. 2). This demonstrates that *D. pachea* NVD can use lathosterol, but not cholesterol, as a substrate (Fig. 1A).

To identify the amino acid changes responsible for the loss of *D. pachea* NVD activity with cholesterol, we reconstructed ancestral NVD sequences (8) for the entire protein region except for the N-terminal region, which does not show conserved amino acid sequence among insects. Interestingly, we found 19 mutations in the lineage leading to *D. pachea*, of which five are predicted to affect protein function (Fig. 1C). We sequenced the entire *nvd* coding region in three *D. pachea* strains and in two natural population samples. The five predicted functionally relevant amino acids were found in all 32 individuals. To test whether these five amino acid changes affect NVD activity, we established an in vitro assay of NVD activity with green fluorescent protein (GFP)—control and NVD constructs (8, 15). GFP-transfected cells produced no 7DHC, whereas cells transfected with *nvd* from various insects, including *D. acanthoptera*, converted cholesterol and lathosterol into 7DHC (Fig. 3A and fig. S6). In accordance with our *D. melanogaster* transgenic assays, we observed that cells transfected with *D. pachea nvd* do not convert cholesterol into 7DHC but do convert lathosterol into 7DHC (Fig. 3A and fig. S6), although at a lower level relative to other species. Control experiments with hemagglutinin epitope-tagged NVD constructs revealed that *D. pachea* NVD accumulates at similar levels as the other NVD homologs in the in vitro assay (fig. S7). These results indicate that

Fig. 3. NVD enzyme activity with cholesterol (left, gray) or with lathosterol (right, white). (A) WT NVD enzymes. (B) *D. mojavensis* NVD enzymes containing single mutations. (C) *D. pachea* enzymes containing reverse mutations. Bars represent average activity, error bars mean $\pm$ SD, and dots data points. Two *D. mojavensis* *nvd* WT constructs were used in our assays. Enzyme activity is indicated as a percentage relative to the NVD activity obtained with a *D. mojavensis* *nvd* construct that includes the *nvd* gene 5' untranslated region (5'UTR) (9). All the *D. mojavensis* constructs tested in (B) contained this 5'UTR. The dotted line indicates *D. mojavensis* NVD WT activity (construct containing the 5'UTR) in (B) and *D. acanthoptera* NVD WT activity in (C).



the ancestral *Drosophila* NVD enzyme was likely able to transform both cholesterol and lathosterol into 7DHC and that NVD has subsequently lost the ability to convert cholesterol in the lineage leading to *D. pachea*. We tested the effect of the five predicted functionally relevant amino acid changes by introducing each mutation individually in the *nvd* sequence from *D. mojavensis*, another cactophilic species endemic to the Sonoran desert, which displayed the highest in vitro NVD activity. With either cholesterol or lathosterol as a substrate, substitution P290C slightly increased the activity, G376T and L330I decreased the activity by half, and substitutions of G250A and E377G reduced the activity to less than 18% of the wild-type (WT) activity (Fig. 3B and fig. S7). We also performed the reciprocal experiment and reintroduced the predicted ancestral amino acid residues into the *D. pachea* NVD sequence. We found that NVD activity close to that of *D. acanthoptera* is not restored by a single amino acid change but by four amino acid changes in concert (Fig. 3C and fig. S7). Corroborating these in vitro results, introduction of a *D. pachea* *nvd* construct containing these four amino acid changes into *D. melanogaster* *nvd* RNAi flies rescues development on food supplemented with cholesterol (Fig. 2). We conclude that two to four mutations

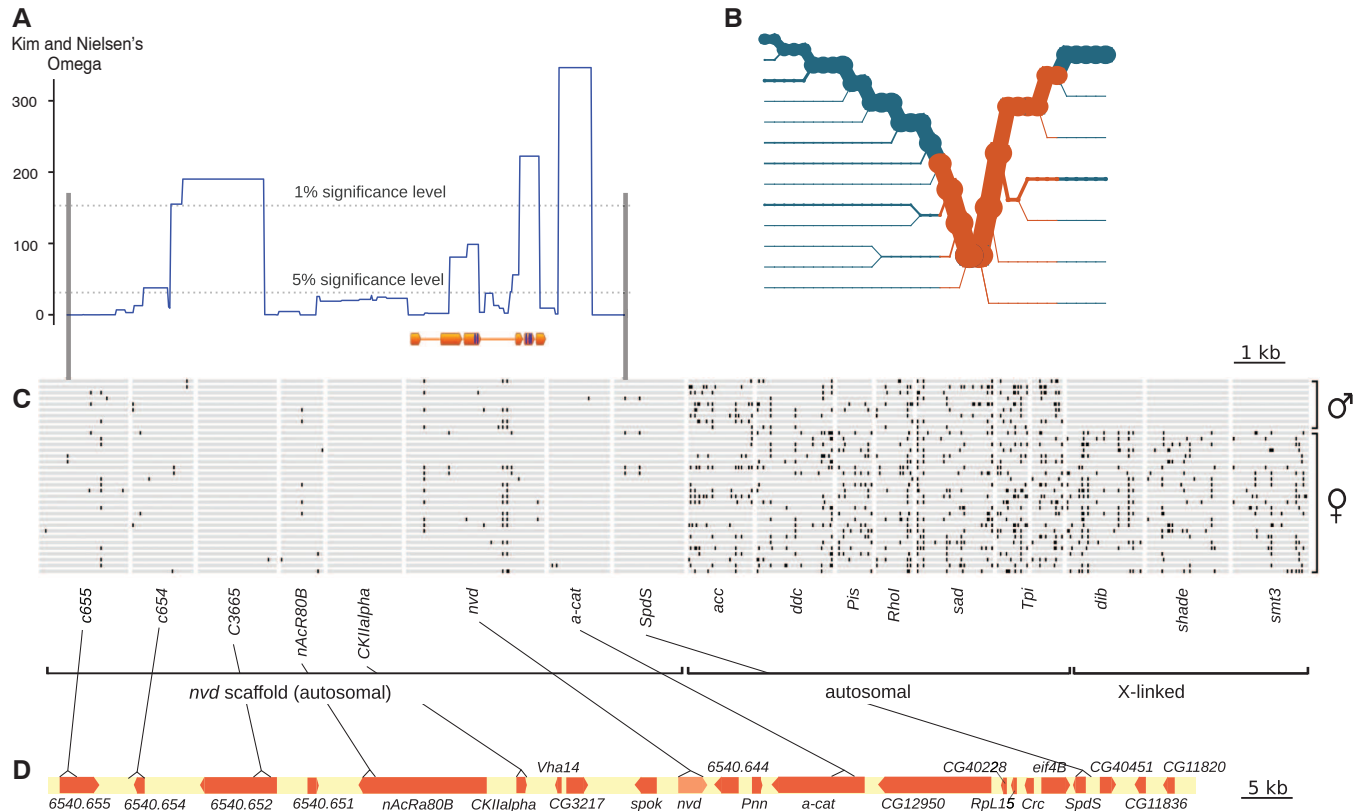


Fig. 4. The *nvd* region is under positive selection. (A) Kim and Nielsen's omega statistic (17) across the *nvd* region. Omega values above the significance level indicate a selective sweep. The *nvd* coding regions are represented below, with the position of the five tested amino acid changes in purple. (B) Haplotype bifurcation plot. Circles indicate polymorphic sites in the *nvd* gene (orange) and in neigh-

boring loci (blue). Line thickness is proportional to the number of samples with the indicated haplotype. (C) Representation of the genotypes of 34 individuals. Black bars indicate heterozygote positions. Homozygote sites for rare alleles are not shown. (D) Position of the sequenced loci within the *nvd* region. Gene annotations are in orange.

in the *D. pachea nvd* coding region have caused the loss of NVD activity with cholesterol substrate. These mutations have turned *D. pachea* into an obligate specialist dependent on lathosterol, a compound that has been found in a single plant species in the Sonoran desert (5, 6).

Remarkably, *D. melanogaster nvd* RNAi flies expressing *D. pachea nvd* survive significantly better on lathosterol than on cholesterol (*t* test, $t_{10,11} = 2.029$, $P < 0.03$) (Fig. 2), but no effect on survival was detected with *nvd* RNAi flies expressing *D. pachea nvd* with the four ancestral amino acid changes (Fig. 2). This suggests that the mutations that abolished cholesterol conversion during *D. pachea* evolution provide a fitness advantage on lathosterol. The underlying mechanism remains unclear. Our in vitro assay does not uncover any benefit from the *D. pachea nvd* mutations: *D. pachea* NVD in vitro activity with lathosterol is not higher compared with other species (Fig. 3), and the NVD enzymes of related *Drosophila* species are already able to convert lathosterol into 7DHC. To assess population genetic forces at play on the *nvd* genomic region, we compared the 3-kb *nvd* locus and seven genes on the same 100-kb scaffold with nine control genes in 34 individuals from a single natural population. Our analysis reveals that *nvd* is in a genomic region of low nucleotide diversity, low recombination rate, and normal divergence rate (McDonald-Kreitman test, $P > 0.85$; maximum likelihood extension of the Hudson-Kreitman-Aguadé test, $P < 10^{-5}$) (Fig. 4 and tables S5 to S11). A signature of a selective sweep is detected [Kim and Nielsen omega (17)] over *nvd* and neighboring loci (Fig. 4), but nucleotide polymorphism is too low to infer whether this recent selection acted on the *nvd* mutations themselves. Tajima's *D* and Fu and Li tests are consistent with recovery from selective sweep in the *nvd* region (table S6).

A likely scenario is that *D. pachea* first evolved a resistance toward senita cactus toxic compounds (5) and slowly became restricted to this food source as it escaped competition with other fly species. Evolution of *D. pachea*'s resistance most likely did not involve NVD because *nvd* is not expressed in the midgut and fat body (fig. S3), the detoxification organs in insects (16). As lathosterol became *D. pachea*'s unique source of sterols for steroid hormone synthesis, mutations in *nvd* that abolished NVD activity on cholesterol appeared and were fixed rapidly due to their beneficial effect with lathosterol. As a result, *D. pachea* became an obligate specialist on the senita cactus. We point out that besides *nvd* mutations, mutation(s) in other genes might also have contributed to *D. pachea* dependence on lathosterol. Alternatively, the identified *nvd* mutations may have spread while *D. pachea* ancestors were still feeding on various plants and may thus have accelerated its ecological specialization. Our study, which uncovered several mutations underlying the obligate bond between a specialist species and its host, illustrates how a

few mutations in a single gene can restrict the ecological niche of a species.

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Supplementary Materials

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Fermentation, Hydrogen, and Sulfur Metabolism in Multiple Uncultivated Bacterial Phyla

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BD1-5, OP11, and OD1 bacteria have been widely detected in anaerobic environments, but their metabolisms remain unclear owing to lack of cultivated representatives and minimal genomic sampling. We uncovered metabolic characteristics for members of these phyla, and a new lineage, PER, via cultivation-independent recovery of 49 partial to near-complete genomes from an acetate-amended aquifer. All organisms were nonrespiring anaerobes predicted to ferment. Three augment fermentation with archaeal-like hybrid type II/III ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) that couples adenosine monophosphate salvage with CO₂ fixation, a pathway not previously described in Bacteria. Members of OD1 reduce sulfur and may pump protons using archaeal-type hydrogenases. For six organisms, the UGA stop codon is translated as tryptophan. All bacteria studied here may play previously unrecognized roles in hydrogen production, sulfur cycling, and fermentation of refractory sedimentary carbon.

Sequencing of total DNA recovered directly from natural systems (metagenomics) often reveals previously unknown genes (1, 2)

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and has the potential to yield near-complete genomes suitable for metabolic and phylogenetic analyses (3–5). Numerous bacteria are known exclusively through cultivation-independent recovery of their ribosomal RNA (rRNA) genes and thus are important targets for this approach (6). Here, we sequenced DNA from three microbial communities from an acetate-amended aquifer to reconstruct genomes of organisms that may contribute to biogeochemical cycling in anoxic

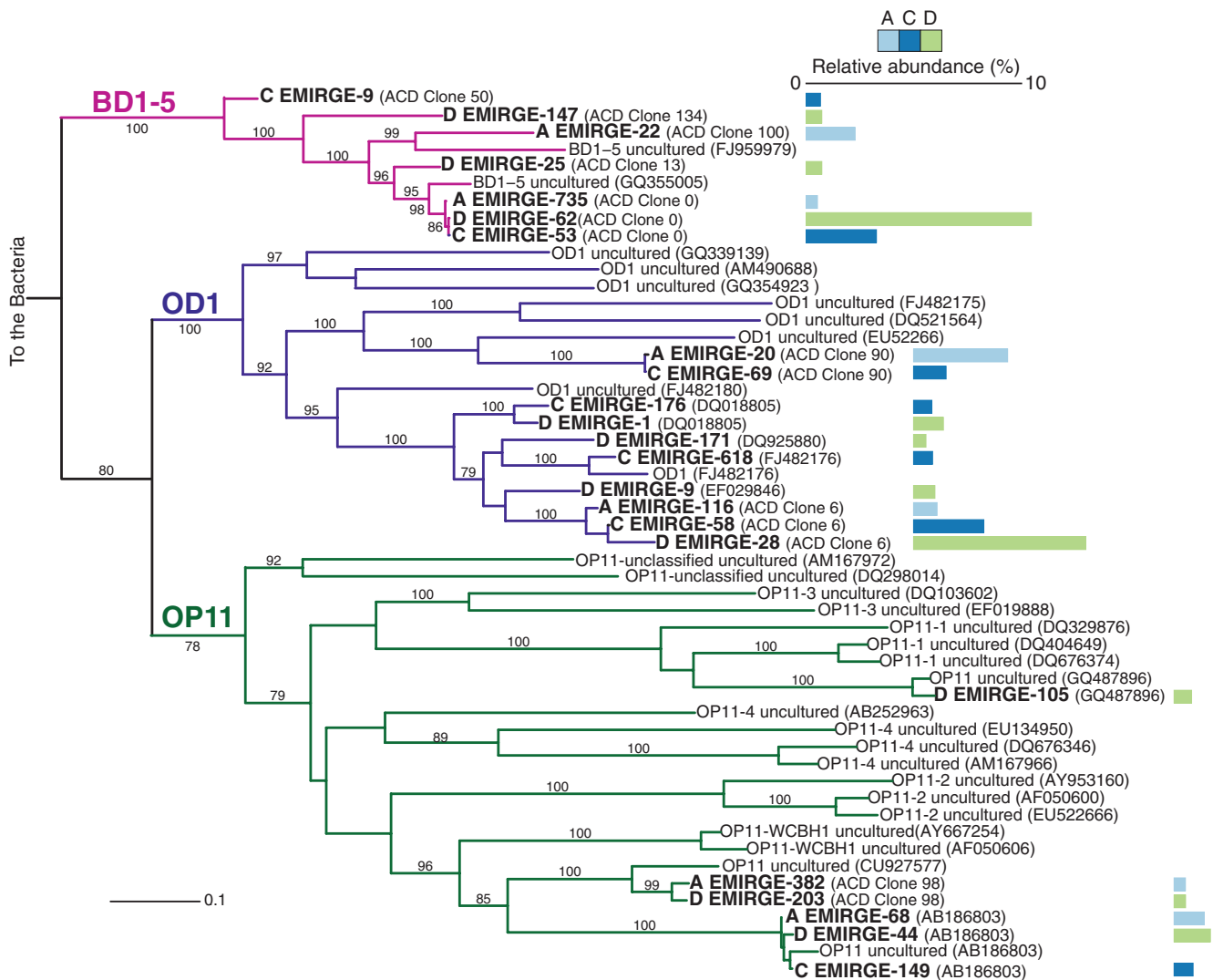


Fig. 1. Maximum likelihood 16S rRNA gene phylogenetic tree showing the placement of the uncultivated phyla recovered by EMIRGE (bold text). The closest representative to each EMIRGE sequence is denoted in parentheses (clone library or Silva accession number). The bar chart

indicates the relative abundance of each sequence in the A, C, and D samples (maximum is 11%), with bootstrap support >80 noted. The 16S rRNA tree from all organisms and details are provided in fig. S2 and (7).

subsurface environments. We recovered 49 genomes from members of candidate phyla widely encountered in rRNA microbial surveys and found evidence for metabolic strategies not previously described in Bacteria. Overall, this study contributes new insights into the physiology and diversity across several major branches of the tree of life.

Groundwater samples (A, C, and D) were collected 5, 7, and 10 days after the start of addition of acetate to an anoxic aquifer in Colorado, USA (fig. S1) (7). From each sample, we recovered microbial cells that passed through a 1.2-μm pre-filter to be retained on a 0.2-μm filter. The samples were immediately frozen on site for DNA extraction and for mass spectrometry-based proteomics to verify the activity of organisms in situ (7).

Illumina sequences from DNA extracted from each sample were coassembled by using strategies optimized for community genomics (7).

Table 1. Overview of genome recovery. Individual genome completion information is in fig. S8.

Candidate divisions	Number of genomes	Percent complete	Bins with >1 genome	Genomes >90%	Genomes >50%	Estimated genes/genome
OD1	21	75 ± 24	0	10	18	1353 ± 350
OP11	19	67 ± 26	5	3	15	1364 ± 252
BD1-5	5	93 ± 3	0	5	5	1540 ± 222
PER	3	80 ± 17	0	2	3	1666 ± 221
ACD80	1	95	0	1	1	1301

Using EMIRGE (8), 16S rRNA genes were reconstructed and confirmed by clone library analysis (7). By linking 16S rRNA (Fig. 1 and fig. S2) to phylogenetically informative genes in the assembly (fig. S3 and table S1), we demonstrated genomic sampling of organisms affiliated with the phylum-level groups OD1, OP11, and BD1-5 (7). Another bacterial group, although it formed a monophyletic clade, did display wandering be-

havior in terms of relative phylogenetic position in protein-coding phylogenetic analyses; it is referred to as the Peregrines (PERs). On the basis of protein-coding trees and partial 16S rRNA gene information, we suggest that PERs may represent a previously unknown phylum-level branch within the bacterial domain (7).

Genome fragments from the coassembly (termed ACD) were clustered into 87 organism



Fig. 2. Detection of alternative coding. **(A)** Histogram of average open reading frame (ORF) length achieved with ORF predictions using the standard bacterial genetic code. The peak with unusually small gene lengths is as-

sociated with ORFs that should have been predicted using code 4. **(B)** Peptides identified by proteomics were mapped onto proteins with code 4 predictions to verify that UGA codes for tryptophan (W).

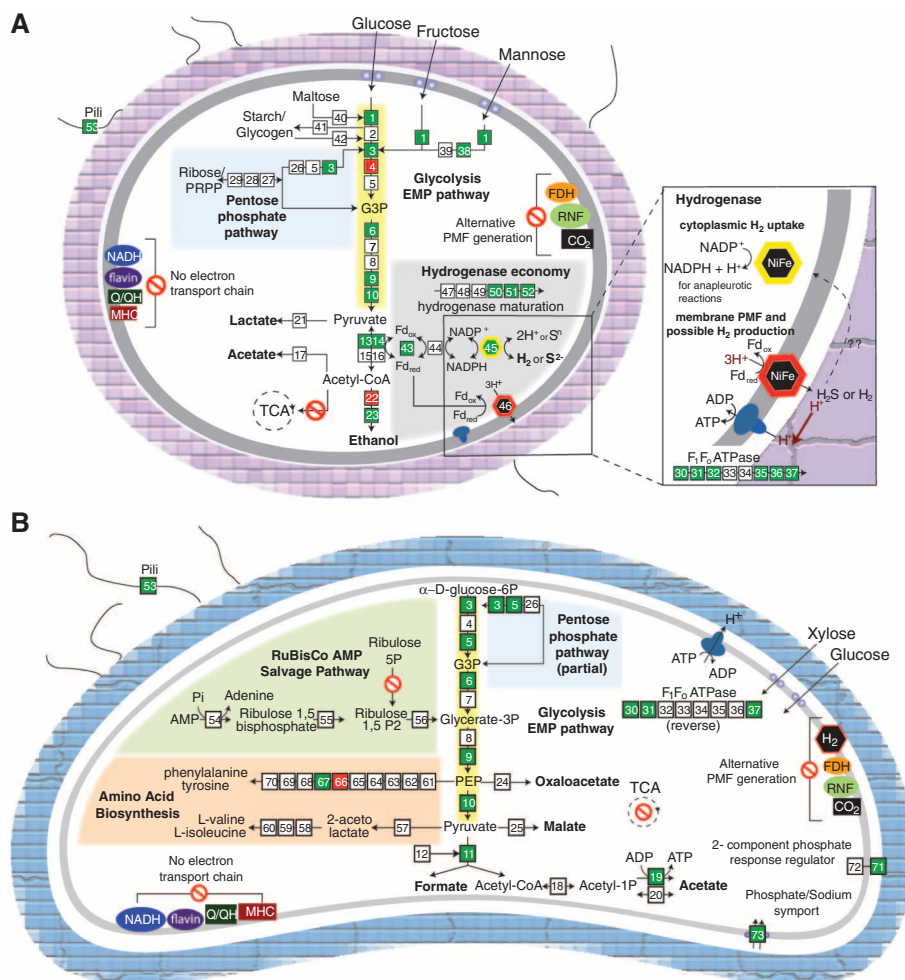


Fig. 3. Metabolic models with detected genes (white box), genes with proteins confirmed by proteomics (green box), and genes missing from pathways (red box). For full gene information for box numbers see table S4. **(A)** OD1-i may produce acetate, ethanol, lactate, and hydrogen as fermentation end products. Hydrogen could be generated via NiFe type 4 membrane-bound hydrogenases (red outline) and cytoplasmic type 3b sulfhydrogenase (yellow outline). The expanded view shows type 4 hydrogenase-generated PMF and ATP synthesis (red line) and possible hydrogen cycling to type 3b hydrogenases (black dashed line). FDH, formate dehydrogenase; Fd_{ox} and Fd_{red} , oxidized and reduced ferredoxin, respectively; G3P, glyceraldehyde-3-phosphate; MHC, multiheme c-type cytochrome; $NADP^+$, nicotinamide adenine dinucleotide phosphate; Q/QH, ubiquinone–reduced ubiquinone; rhodobacter nitrogen-fixing (RNF) complex. **(B)** PERs produce acetate and formate from pyruvate (no formate dehydrogenases or hydrogenases were identified). PER genomes lack detectable mechanisms for recovering additional energy via membrane-potential coupled to ATP synthase but may use RuBisCO, analogous to Archaea, for salvaging ATP by shunting nucleotides through central-carbon metabolism. PEP, phosphoenolpyruvate.

“bins” from emergent self-organizing map analysis of their tetranucleotide sequence composition (9). Organism abundance ratios between samples were used to further refine binning (7). Here, we focus on 49 genomes from BD1-5, OP11, OD1, and PER, relevant to carbon, sulfur, and hydrogen cycling (additional analyses of these and other genomes will be reported separately). From the inventory of conserved, single-copy genes (7, 10), we estimated that 21 of the 49 genomes were >90% complete (Table 1). Note that the majority of these organisms each represented ~1% of the assembled community (table S2).

Previously, only 33 protein-coding genes have been reported for the OD1 phylum (11). We recovered more than 24,000 OD1 gene sequences (with an average of 1119 genes per genome) for 21 species, on genome fragments up to 358 kilobase pairs. Phylogenetically, we resolved multiple OD1 lineages and recognized one sublineage, OD1-i (fig. S3). For OP11, there has only been one fragmented partial sampling (~270 kilobases) from a single cell (16S rRNA OP11 class “unclassified”) (12). Here, we recovered more than 25,000 genes (with an average of 1337 genes per genome) for 19 organisms from OP11 classes I and WCBH1-64 (Fig. 1). There has been no previous genomic sampling of PER or BD1-5 (7).

BD1-5 genes predicted using the standard bacterial genetic code were anomalously short (7), with a low overall coding density. We deduced, and confirmed using proteomic analyses (Fig. 2), that they use genetic code 4, where the normal stop codon (UGA) is translated as tryptophan (W). Recoding of UGA to W in Bacteria is rare but has been noted within the Firmicutes and Proteobacteria phyla (some Mollicutes and Alphaproteobacteria). It is often associated with small genomes and low GC content and may be a consequence of genome reduction (13). Our code 4 genomes are estimated to be <2 Mb and have low but variable GC contents (27 to 43%). One genotype, ACD80 is phylogenetically very distantly related to the other BD1-5 organisms (fig. S3), and it may ultimately be recognized as a separate lineage (7). Future sampling may resolve whether code 4 usage is an ancestral trait or arose independently.

Genomic analyses indicate that all 49 organisms were nonrespiring. Given the lack of a tricarboxylic acid (TCA) cycle, subunits of the reduced form of nicotinamide adenine dinucleotide (NADH) dehydrogenase, and most other electron-transport chain complexes including terminal oxidases, we infer a strictly anaerobic fermentation-based lifestyle. All have a glycolysis (Embden-Meyerhof-Parnas) pathway and convert pyruvate to acetyl-coenzyme A (acetyl-CoA) without pyruvate dehydrogenase, instead they use pyruvate-formate lyase (PFL in PER) or pyruvate ferredoxin oxidoreductase (PFOR in OD1, OP11, and BD1-5) (Fig. 3). Most generate adenosine triphosphate (ATP) by converting acetyl-CoA to acetate via two enzymes (acetate kinase and phosphate acetyltransferase) (Fig. 3B). However, the OD1-i are predicted to use a single enzyme, acetyl-CoA synthetase (EC: 6.2.1.13) (Fig. 3A) for ATP generation, which is common in some fermentative sulfur-reducing Archaea (e.g., *Pyrococcus* spp.) but rare in Bacteria (14). OD1-i may reoxidize NADH produced during glycolysis by converting pyruvate to D-lactate and acetyl-CoA to ethanol (Fig. 3A). Excreted fermentation end products could sustain Geobacteraceae and sulfate-reducing bacteria that bloom when acetate is added to the aquifer to promote uranium bioremediation (fig. S2) (15).

The BD1-5, OP11, and OD1 phyla contain members that augment substrate-level phosphorylation from fermentation by coupling a mem-

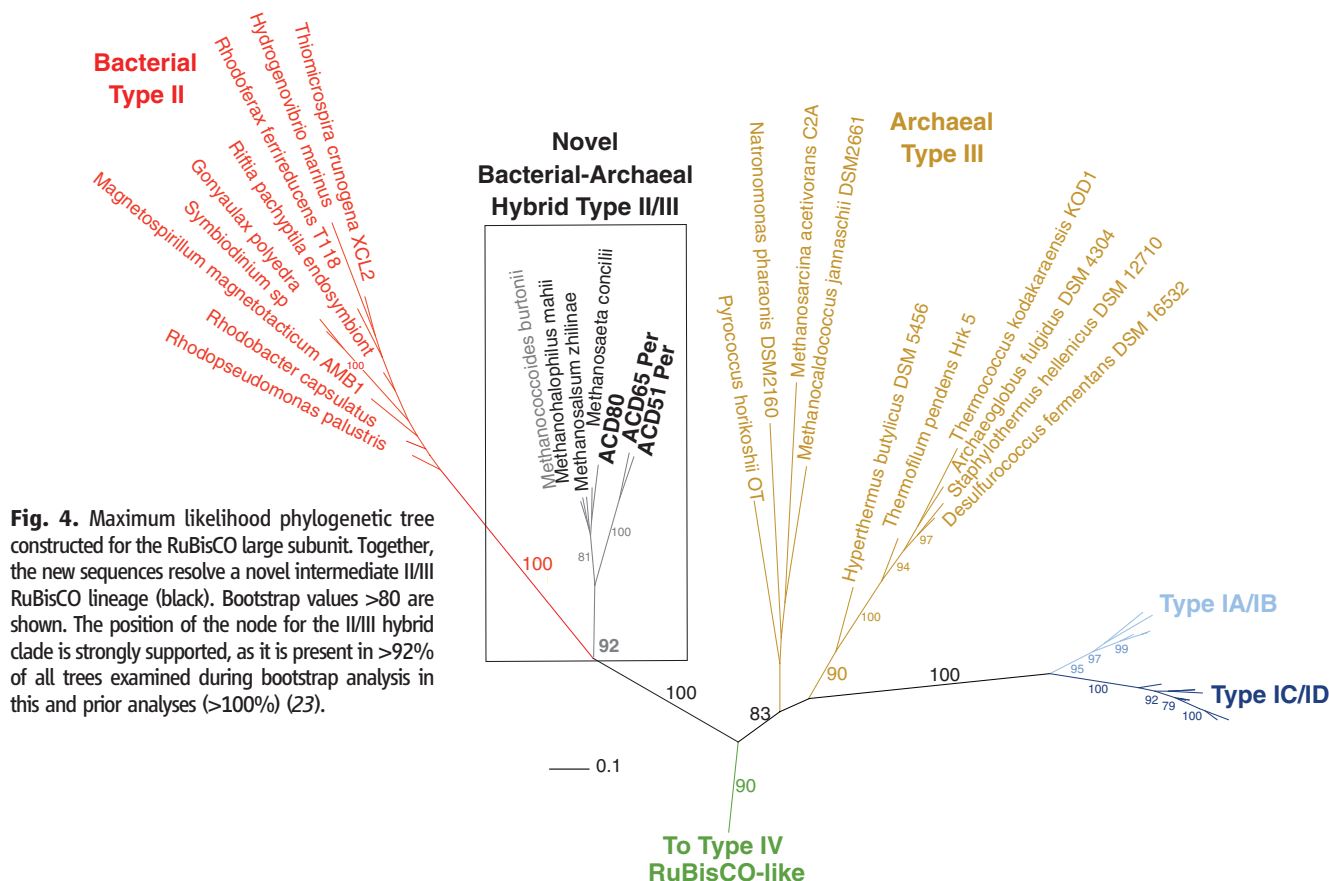
brane proton-motive force (PMF) (independent of a canonical electron-transport chain) to ATP generation via a F_1F_0 ATP synthase. In some BD1-5 and OP11, this appears to rely on proton-pumping by membrane-bound pyrophosphatases (H^+ -PPases). Similar H^+ -PPases studied in syntrophic bacteria (16) translocate protons across the membrane for energy conservation via ATP synthase. We find proteomic support for H^+ -PPase (BD1-5 and OP11), alcohol dehydrogenase (OD1), glycolysis, PFOR or PFL, and ATP synthase from all lineages; this confirmed fermentation in situ (Fig. 3 and table S3).

Some fermentative anaerobes produce H_2 to dispose of excess reductant (17). In OD1 and OP11, we identified three Fe-only hydrogenases and 23 NiFe hydrogenases (table S5). Phylogenetic analyses of the NiFe hydrogenase catalytic subunits revealed that 17 are type 3b cytoplasmic hydrogenases most closely related to those of fermentative, sulfur-reducing Thermococcales Archaea (figs. S4 and S5) (18). The type 3b hydrogenases may produce H_2 during fermentation or H_2S when polysulfide is available. Alternatively, they may consume H_2 to produce the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) for anabolic metabolism (18, 19). Proteomic detection of hydrogenase-related proteins in conjunction with proteins for fermentation, as well as the abundance of organisms with this capacity when sulfide was detected in groundwater (Figs. 1 and 3A), supports a role in H_2 or H_2S

generation rather than H_2 consumption. We also identified type 4 membrane-bound hydrogenase genes in some OD1 genomes (fig. S5). The dual hydrogenase system may function in intracellular hydrogen cycling, as in Thermococcales (7, 20–22), where PMF and H_2 are produced by the membrane-bound hydrogenase, and H_2 is shuttled to the cytoplasmic hydrogenase where it is oxidized to produce NADPH (Fig. 3A).

In ACD80 and two PER genomes, we identified putative ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) genes potentially involved in CO_2 assimilation. All residues for catalytic activity are present, except one for substrate binding that is absent in the ACD80 version (fig. S6). This finding and structural modeling (7) suggest carboxylase-oxygenase activity. Our RuBisCO sequences clade separately from the bacterial type II sequences and are most closely related to a sequence from *Methanococcoides burtonii* (MBR) and a global ocean sampling (GOS)-derived sequence of unknown affiliation that may represent a II/III hybrid RuBisCO (Fig. 4) (23). We expanded the membership of this clade to eight sequences by identifying three genes in publicly available methanogenic archaeal genomes (*Methanohalophilus zhilinae*, *Methanosaeta concilii*, and *Methanohalophilus mahii*) (table S1C). Our data show that the RuBisCO hybrid occurs in Bacteria.

The MBR type II/III RuBisCO function is comparable to the type III archaeal RuBisCO (23). It does not function in the Calvin-Benson-



Bassham (CBB) pathway but fixes CO₂ and contributes to adenosine monophosphate (AMP) recycling (7, 24). We predict that the bacterial type II/III RuBisCO genes share this function. Homologs of DeoA and E2b2, key enzymes of the CO₂-fixing AMP-recycling pathway (24), were identified (Fig. 3B), and no essential CBB cycle enzymes (e.g., phosphoribulokinase) were detected. Salvaging purine-pyrimidine products to produce RuBisCO generates 3-phosphoglycerates and perhaps pyruvate (ACD80), which could ultimately be fermented for ATP production (7).

From this study and rRNA gene survey information indicating prevalence in anoxic, organic carbon-rich environments (25, 26), we predict widespread fermentation-based metabolism in the 49 OD1, OP11, BD1-5, and PER genomes sampled here. We find it intriguing that several pathways for anoxic carbon, hydrogen, and sulfur cycling in these organisms share features previously documented only in Archaea. Some OD1 may contribute to sulfur cycling, on the basis of their previous association with sulfur-rich environments (11, 12, 26–28). Given the absence of genes for sulfur respiration in our near-complete OD1-i genomes, the link may involve hydrogenase-mediated sulfur-reductase activity. Notably, these insights were obtained through cultivation-independent analyses and have contributed more near-complete (>90%) genomic sampling for OD1 than is available for almost half of all of the genomically characterized bacterial phyla (29).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6102/1661/DC1
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Supplementary Text
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Database 1

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Disulfide Rearrangement Triggered by Translocon Assembly Controls Lipopolysaccharide Export

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The presence of lipopolysaccharide (LPS) on the cell surface of Gram-negative bacteria is critical for viability. A conserved β -barrel membrane protein LptD (lipopolysaccharide transport protein D) translocates LPS from the periplasm across the outer membrane (OM). In *Escherichia coli*, this protein contains two disulfide bonds and forms the OM LPS translocon with the lipoprotein LptE. Here, we identified seven *in vivo* states on the oxidative-folding pathway of LptD. Proper assembly involved a nonfunctional intermediate containing non-native disulfides. Intermediate formation required the oxidase DsbA, and subsequent maturation to the active form with native disulfides was triggered by LptE. Thus, disulfide bond-dependent protein folding of LptD requires the proper assembly of a two-protein complex to promote disulfide bond rearrangement.

A defining feature of Gram-negative organisms is the presence of lipopolysaccharide (LPS) on the cell surface (1). LPS must be properly assembled in the outer leaflet of the outer membrane (OM) to establish a permeability barrier against toxic compounds, including antibiotics (2, 3). In *Escherichia coli*, a translocon responsible for LPS movement across the OM is composed of two essential OM proteins: an in-

tegral β -barrel protein, LptD (lipopolysaccharide transport protein D), and a lipoprotein, LptE (4–6). The LptD/E complex forms part of the trans-envelope LPS exporter, which contains five other essential Lpt proteins that collectively move LPS from the inner membrane (IM) to the cell surface (7). Assembly of the OM LPS translocon presents a challenging protein-folding problem, because LptE resides inside LptD (5, 6) and

formation of the correct disulfide bonds in LptD is required for this translocon to function (8). How the cell coordinates assembly of the OM complex with the formation of the rest of the trans-envelope exporter is unknown.

To understand the assembly of the functional OM LPS translocon, we examined the biogenesis of LptD. *E. coli* LptD contains an N-terminal periplasmic domain (amino acids 25 to 202) and a C-terminal integral β -barrel domain (amino acids 203 to 784) (5), which is folded and inserted into the OM by the Bam complex (β -barrel assembly machine) (9–11). LptD has four cysteine residues, two in the N-terminal domain (Cys³¹ and Cys¹⁷³) and two very near the C terminus (Cys⁷²⁴ and Cys⁷²⁵). In its mature form, LptD contains two long-range nonconsecutive disulfide bonds connecting the N- and C-terminal domains, one

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between the first and third cysteines (Cys³¹-Cys⁷²⁴; [1-3]) and the other between the second and fourth cysteines (Cys¹⁷³-Cys⁷²⁵; [2-4]) (Fig. 1A) (8). Either of these two disulfides is sufficient for LptD to function (8).

LptE is required to promote assembly of mature LptD (8). We wondered if an LptD intermediate would accumulate in vivo when LptE is the limiting reagent. OM fragments isolated from wild-type (WT) cells contain only mature LptD, which migrates more slowly than reduced LptD during SDS-polyacrylamide gel electrophoresis (PAGE) (Fig. 1A) (8, 12, 13). In contrast, OM fragments from an LptE-limiting strain, which produced lower levels of LptE (fig. S1), contained a new LptD species. In the absence of β-mercaptoethanol, this species migrated slightly faster than fully reduced LptD, suggesting that it was disulfide-bonded (Fig. 1A). To assign the disulfide connectivity of this LptD species (intermediate 1), we examined the SDS-PAGE migration patterns of the six LptD mutant proteins in which two of the cysteines were changed to serine residues (8). Only the mutant lacking the last two cysteines (LptD_{CCSS}), which can make the Cys³¹-Cys¹⁷³ ([1-2]) disulfide bond within the

N-terminal domain, exhibited faster mobility than reduced LptD (fig. S2). Thus, the LptD intermediate 1 that accumulates when LptE is limiting contains the consecutive [1-2] disulfide bond, and LptE is required for the conversion of this intermediate to LptD with its native disulfide bonds. Whether the third and fourth cysteines are ever disulfide-bonded in this intermediate remains unclear, although we have obtained some evidence suggesting that the [3-4] disulfide bond may be formed (fig. S3).

To examine how defects in either component affect translocon assembly, we analyzed three mutants: LptD_{Δ330-352} (LptD4213) (14), LptD_{Δ529-538} (6), and LptE_{Δ100-101/P99R} (LptE6) (15). Two of these mutations impair interactions between LptD and LptE (6, 15). Although none of these mutations lowered the levels of LptE in the OM, each resulted in substantial accumulation of intermediate 1 ([1-2]-LptD) (Fig. 1B). LptD containing a [1-2] disulfide bond is not functional (8), which explains why these mutants have defective OMs (6, 14, 15).

To determine whether intermediate 1 is a dead-end product or on the folding pathway of LptD in a WT strain, we pulse-labeled FLAG₃-tagged LptD

with [³⁵S]-methionine and monitored its progression to the mature form during a cold-methionine chase. The predominant form at the start of the chase was [1-2]-LptD (intermediate 1), which was slowly converted to mature [1-3][2-4]-LptD (Fig. 2A). The final distribution of LptD species at ~20 min was identical to that in the steady state (fig. S4, A and B). Thus, a non-native species with a disulfide bond (intermediate 1) is an intermediate along the oxidative-folding pathway of LptD in vivo. Furthermore, formation of mature [1-3][2-4]-LptD proceeds via disulfide bond rearrangement.

We next performed pulse-chase experiments under conditions that preserve a folded β barrel to determine whether disulfide bond rearrangement occurs before or after β-barrel folding. When samples were not heated before SDS-PAGE, mature [1-3][2-4]-LptD migrated as a broad band with faster gel mobility than the heat-denatured form, indicating that the β barrel was folded (Fig. 2B) (5). The amount of folded [1-3][2-4]-LptD increased with time. A new, fast-migrating LptD species, appearing at the start and chasing away by 20 min, could be detected (Fig. 2B). This species was also heat-modifiable and ran at the same position as the major band in a nonheated LptD_{CCSS} sample containing the [1-2] disulfide (Fig. 2B). We assign this LptD species as intermediate 1 containing a folded β barrel. Because folded intermediate 1 chased to folded mature [1-3][2-4]-LptD, we conclude that the LptD β barrel folds before disulfide rearrangement occurs. Furthermore, monitoring protease susceptibility supported this notion (fig. S5). Because this [1-2]-LptD species did not accumulate substantially during the chase, we conclude that folding is slower than rearrangement.

LptD is a substrate of the periplasmic oxidase DsbA (16), and oxidation of LptD is impaired in strains lacking DsbA (fig. S4) (8, 12). We sought to elucidate the role of DsbA in introducing disulfide bonds in LptD by using the DsbA_{P151T} mutant that forms kinetically stable mixed-disulfide intermediates with substrate proteins to see if we could accumulate cross-linked adducts between LptD and DsbA (16). We observed two LptD species that also contained

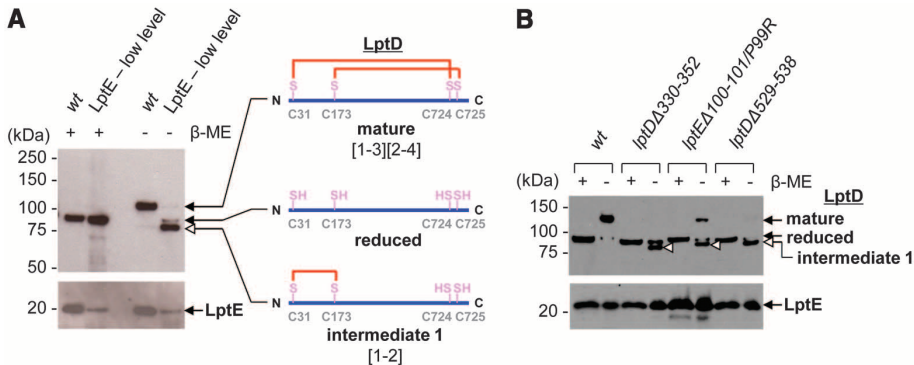
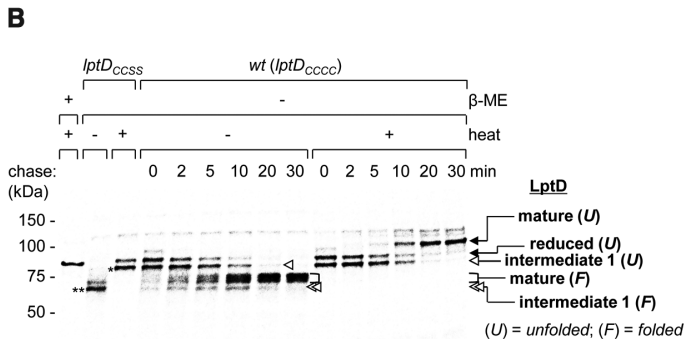


Fig. 1. A non-native LptD intermediate with a disulfide bond (1, [1-2]-LptD) accumulates in strains with defective LptD or LptE. (A) α-LptD and α-LptE immunoblot analyses of OM fragments obtained from wild-type (wt) and LptE-limiting strains grown to early log phase (see fig. S1). More membranes (3×) from cells expressing limited LptE were loaded. (B) α-LptD and α-LptE immunoblot analyses of OM fragments obtained from wt, *lptD*_{Δ330-352}, *lptE*_{Δ100-101/P99R}, and *lptD*_{Δ529-538} strains. White arrowheads indicate the position of [1-2]-LptD in each strain. Where indicated, β-mercaptoethanol (β-ME) was used to reduce disulfide bonds.

Fig. 2. Intermediate 1 is observed along the LptD assembly pathway in vivo. Folding of the LptD β-barrel domain is slow and precedes disulfide bond rearrangement. (A) WT cells expressing LptD-FLAG₃ were pulsed with [³⁵S]-methionine and chased with cold methionine. The samples were alkylated with *N*-ethylmaleimide, immunoprecipitated with α-FLAG antibody, and analyzed by SDS-PAGE/autoradiography. (B) [³⁵S]-Met pulse-chase of newly synthesized LptD-FLAG₃, where samples were processed under nondenaturing conditions and analyzed



by semisynthetic SDS-PAGE to preserve (- heat) or denature (+ heat) the β barrel. Pulse-labeled LptD_{CCSS}-FLAG₃ marks the positions of unfolded (single asterisk) and folded (double asterisks) [1-2]-LptD.

DsbA (Fig. 3A). We then identified these new bands as mixed-disulfide intermediates linking DsbA to Cys³¹ of reduced LptD (DsbA adduct A; [1-DsbA]) and to Cys³¹ of [2-4]-LptD (DsbA adduct B; [2-4][1-DsbA]) by comparing adducts observed with LptD using Cys-to-Ser mutants (Fig. 3, A and C, and fig. S6). DsbA adduct A was likely to correspond to the intermediate that first formed between LptD and DsbA, leading to the formation of the [1-2] disulfide bond (Fig. 3C). When we performed pulse-chase analysis in the *dsbA_{P151T}* strain, we detected [³⁵S]-labeled DsbA adduct A at early time points ($t = 0$ to 2 min) (Fig. 3B), consistent with this interpretation. We also observed the appearance of DsbA adduct B ([2-4][1-DsbA]-LptD) at $t = 2$ min, which chased by $t = 40$ min. The fact that this species appeared before (and chased into) [1-3][2-4]-LptD suggests that DsbA adduct B is also along the assembly pathway and that DsbA directly introduces the [1-3] disulfide bond in LptD after an intermediate containing the [2-4] disulfide bond (intermediate 2, see fig. S4) is already formed (Fig. 3C). Thus, DsbA makes both the [1-2] and [1-3] disulfide bonds in LptD at different stages of assembly.

The ability to detect several DsbA adducts with LptD during pulse-chase analysis allowed us to describe the sequence of events leading to the assembly of a functional OM LPS translocon.

We could assign seven distinct species along the oxidative-folding pathway of LptD in vivo (Fig. 3C). The first nonconsecutive disulfide bond formed between Cys¹⁷³ and Cys⁷²⁵ ([2-4]) via disulfide bond rearrangement from [1-2]-LptD. Because either of the two nonconsecutive disulfide bonds is sufficient for the proper function of LptD (8), the [2-4]-LptD species (intermediate 2) represents the first functional form of LptD. In this regard, it is important to point out that both Cys¹⁷³ and Cys⁷²⁵ are present in >95% of more than 1000 Gram-negative LptD proteins that have nonidentical sequences (fig. S7) (12). In contrast, Cys³¹ and Cys⁷²⁴ are much less conserved, suggesting that the [2-4] disulfide bond plays a critical structural role in the function of the translocon.

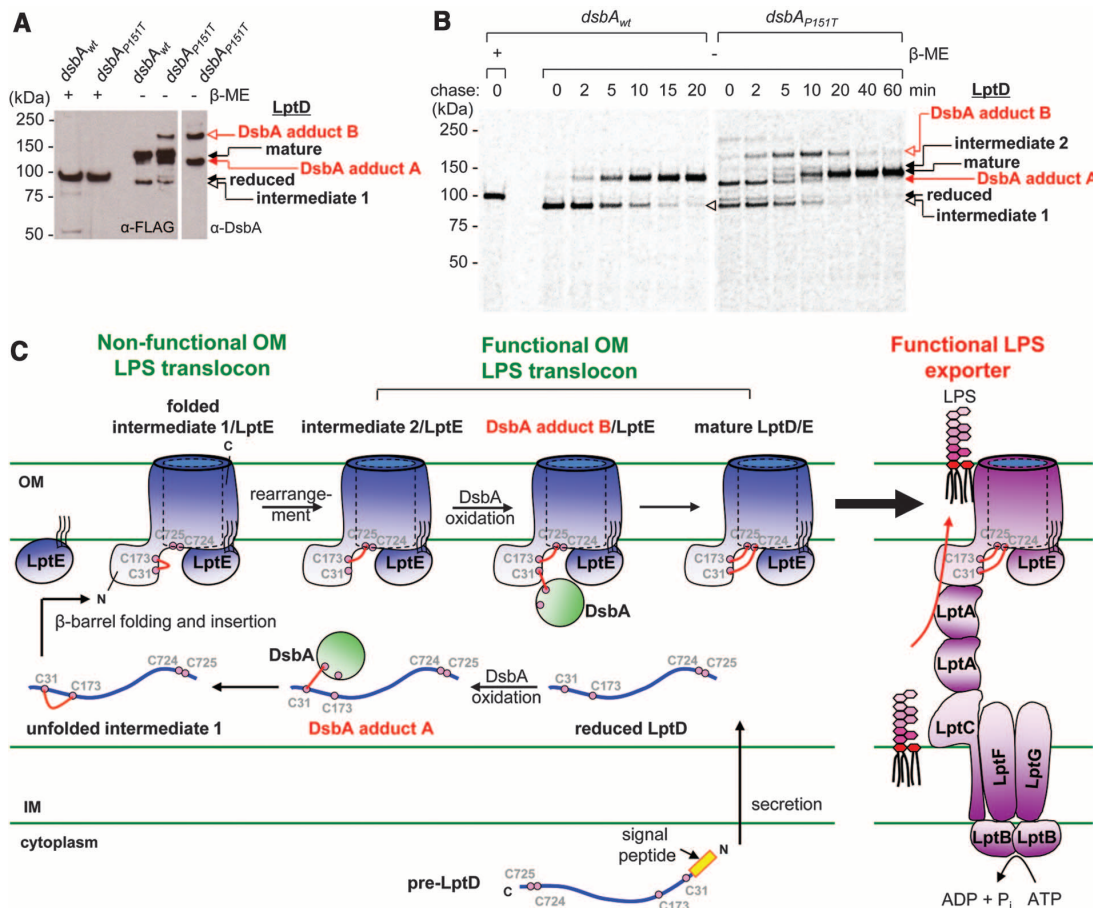
LptD and LptE function together in a single step to insert LPS correctly on the cell surface (4–6). The progression of LptD from an unfolded intermediate to a preassembled nonfunctional complex with LptE containing non-native disulfide bonds (intermediate 1), and then to a rearranged functional state containing native disulfide bonds, provides a mechanism to check that both translocon components have been properly synthesized, targeted, and assembled together in the OM. Mutants in LptD or LptE accumulate intermediate 1 (Fig. 1B), suggesting that assembly of the translocon stalls in a nonfunctional state if there are defects in either component. Interme-

diate 1 also accumulates in strains with limited LptE (Fig. 1A), but it can be completely converted to the functional form of LptD if additional LptE is subsequently expressed (fig. S8). This “rescue” experiment demonstrates that increased LptE levels, which permit formation of the plug-and-barrel architecture, trigger rearrangement of the disulfide bonds. Thus, assembly of the LptD/E complex controls activation of the translocon; only when the complex is properly formed does disulfide bond rearrangement occur efficiently (Fig. 3C). The oxidative assembly of LptD is regulated by disulfide bond rearrangement that is triggered by binding to LptE, which, in fact, is not even an oxidoreductase.

Producing nonfunctional intermediate 1 before activation via disulfide bond rearrangement also provides a mechanism for quality control to ensure that the trans-envelope LPS exporter is assembled with only a functional OM translocon (Fig. 3C). Proper disulfide bonds in LptD are required for the trans-envelope Lpt bridge to form via interactions between LptA and LptD (17). The triggering strategy prevents a defective OM translocon from achieving the correct disulfide bond configuration, thereby preventing formation of a nonfunctional trans-envelope LPS exporter that might release LPS into an inappropriate compartment.

Our ability to visualize and accumulate different oxidation states of LptD allows us to extend

Fig. 3. The oxidative-folding pathway of LptD. (A) LptD intermediates containing mixed-disulfide adducts with DsbA can be detected in vivo. α -FLAG immunoprecipitation using *wt* or *dsbA_{P151T}* cells containing pET23/42/*lptD*-FLAG₃. Samples were analyzed by α -FLAG and α -DsbA immunoblots. (B) [³⁵S]-Met pulse-chase of newly synthesized LptD-FLAG₃ in *wt* and *dsbA_{P151T}* backgrounds. (C) Biogenesis of the trans-envelope LPS exporter. The six intermediate LptD species observed experimentally are depicted as they appear after secretion across the IM during oxidative assembly of the OM translocon. ADP, adenosine diphosphate; P_i, inorganic phosphate; ATP, adenosine triphosphate.



to membrane proteins the classic approach of using disulfide bonds to study the folding of soluble proteins in vivo (18, 19). Our studies have established that the rate-determining step in translocon assembly is β -barrel folding. Folding is remarkably slow, taking ~20 min, or one-third of the cell cycle (Fig. 2). The porin LamB folds several orders of magnitude faster than LptD (20). LptD folding may be slow because the protein must wrap around LptE, forming a β barrel that also contains a plug (5, 6). Given the slow rate of LptD folding, it may be possible to design inhibitors against the long-lived nonfunctional states of the OM LPS translocon, thus preventing its activation.

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Sedlin Controls the ER Export of Procollagen by Regulating the Sar1 Cycle

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Newly synthesized proteins exit the endoplasmic reticulum (ER) via coat protein complex II (COPII) vesicles. Procollagen (PC), however, forms prefibrils that are too large to fit into typical COPII vesicles; PC thus needs large transport carriers, which we term megacarriers. TANGO1 assists PC packing, but its role in promoting the growth of megacarriers is not known. We found that TANGO1 recruited Sedlin, a TRAPP component that is defective in spondyloepiphyseal dysplasia tarda (SED), and that Sedlin was required for the ER export of PC. Sedlin bound and promoted efficient cycling of Sar1, a guanosine triphosphatase that can constrict membranes, and thus allowed nascent carriers to grow and incorporate PC prefibrils. This joint action of TANGO1 and Sedlin sustained the ER export of PC, and its derangement may explain the defective chondrogenesis underlying SED.

Newly synthesized protein cargoes are exported from the endoplasmic reticulum (ER) in transport carriers. Cargo sorting and carrier budding occur at ER exit sites (ERES) and are mediated by the small guanosine triphosphatase (GTPase) Sar1, which recruits the COPII coat complex (1). Procollagen (PC), however, forms 300-nm triple helices whose size exceeds that of typical 60- to 90-nm COPII vesicles. Although there has been important progress in identifying receptors such as TANGO1 that guide the packing of PCs into COPII transport carriers (2–4), the growth promotion mechanism of such megacarriers is unknown, as is the nature of the timer that delays fission of the nascent carriers until they reach an adequate size.

Spondyloepiphyseal dysplasia tarda (SED) is an X-linked skeletal disorder characterized by short stature, a short trunk, and precocious

osteoarthritis. SED is caused by mutations in the gene *SEDL* (5); the underlying defect is a derangement in chondrogenesis reflecting the inability of chondrocytes to properly secrete extracellular matrix components (6). Sedlin, the *SEDL* product, is a component of a highly conserved multisubunit complex, TRAPP, which functions at various steps in intracellular transport (7), but the role of Sedlin (also called TRAPPC2) in these processes remains unknown.

The observations that a defect in Sedlin leads to cartilage-restricted consequences, and that there are shared clinical signs between patients with some mutations of type II PC (PCII) (8, 9) and Sedlin (5), prompted us to examine the impact of Sedlin knockdown (KD) (fig. S1) on trafficking of PCII in chondrocytes (10) and, in comparison, on trafficking of a reporter cargo, the temperature-sensitive variant of vesicular stomatitis virus G

protein (ts045–VSV-G). The transport of PCII and VSV-G along the secretory pathway was synchronized by incubating cells at 40°C, resulting in reversible misfolding and ER retention of both cargoes, and then shifting the cells to 32°C to release the ER block (11). PCII transport was impaired by Sedlin depletion, with a marked inhibition of its ER exit (Fig. 1, A and B, and fig. S2A) and extracellular secretion (Fig. 1C). This impairment was partially rescued by expression of an RNA-resistant form of Sedlin (Fig. 1B). By contrast, transport of newly synthesized VSV-G was indistinguishable in Sedlin-depleted cells relative to control cells (Fig. 1, A and B, and fig. S2B). Sedlin KD did not appreciably affect total protein secretion (Fig. 1D). We extended our analysis to the transport of other cargoes, including type I PC (PCI), CD8 α (Fig. 1E), albumin, and α 1-antitrypsin (Fig. 1F). PCI was the only cargo whose ER exit was affected by Sedlin depletion. All other cargoes examined exited the ER and reached the plasma membrane at apparently normal rates. Sedlin plays its role in PC trafficking as a TRAPP component, because a similar selective impairment of the ER exit of PC was induced by depleting the whole TRAPP by knockdown of

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Bet3 (TRAPPC3), a core component of the complex that, when depleted, destabilizes TRAPP (12) (fig. S1A and figs. S3 to S5). We then analyzed fibroblasts from SEDT patients (fig. S6). During the 40°C block, mCherry-PCII was retained in the ER in both wild-type and SEDT cells. After 30 min at 32°C, mCherry-PCII was almost completely concentrated in the Golgi complex in wild-type cells but remained in the ER in SEDT cells (Fig. 1G and fig. S6). Expression of wild-type Sedlin in SEDT fibroblasts partially rescued the ER export of mCherry-PCII (Fig. 1G).

The requirement of Sedlin for the ER exit of PC suggested that it might play a role at the ERES. Indeed, Sedlin associated with ERES (Fig. 2A), as shown for Bet3 (7). Sedlin was massively recruited to ERES during a synchronized wave of PCII transport (Fig. 2, B and C). We wondered how Sedlin, a cytosolic protein, could sense the presence of PC in the ER lumen. Because the transmembrane protein TANGO1 is a PC receptor at ERES (2, 3), we investigated whether recruitment of Sedlin to ERES involved TANGO1. TANGO1 overexpression increased Sedlin association with ERES (Fig. 2D) and also accelerated the ER exit of PC (fig. S7). Conversely, TANGO1 depletion (fig. S1B) decreased Sedlin association with ERES (Fig. 2E). We next explored the molecular mechanisms underlying TANGO1-

mediated recruitment of Sedlin to the ERES and found that Sedlin and TANGO1 physically interacted (Fig. 2F).

The above findings prompted us to investigate the role of Sedlin at ERES. Sedlin depletion slowed the COPII coat cycle because it prolonged the recovery time of green fluorescent protein (GFP)-tagged Sec23 fluorescence recovery after photobleaching (FRAP) (Fig. 2G) and slowed the dissociation of GFP-Sec23 from ERES membranes, as assessed by fluorescence loss in photobleaching [dissociation rate (K_{off}) values, 0.032 s^{-1} in control cells, 0.022 s^{-1} in Sedlin KD cells]. GFP-Sec23 exhibited slower dynamics in SEDT fibroblasts relative to wild-type fibroblasts as well, and the expression of wild-type Sedlin in SEDT fibroblasts rescued the COPII cycling defect (Fig. 2H).

In addition to COPII coat components, Sar1 was also more stably associated with membranes in Sedlin-depleted cells relative to control cells (Fig. 3A). Because Sar1 associates with membranes in its GTP-bound state, we tested the levels of Sar1-GTP in Sedlin-depleted cells. Using an antibody specific for Sar1-GTP, we observed higher levels of Sar1-GTP in Sedlin-depleted cells than in control cells (Fig. 3B and fig. S8). Slower COPII cycles and increased Sar1-GTP levels similar to those caused by Sedlin depletion were detected in Bet3/TRAPP-depleted cells (fig.

S9), indicating that Sedlin acts to control the active state of Sar1 as a TRAPP component.

The increased Sar1-GTP levels observed after Sedlin depletion implied that ER exit of PC and VSV-G might be differentially sensitive to Sar1-GTP. We tested this hypothesis by evaluating the impact of microinjecting increasing amounts of the GTP-locked mutant of Sar1 (His⁷⁹ → Gly; Sar1H79G) on the ER exit of PC and VSV-G. At a low level of Sar1H79G (0.1 mg/ml), ER exit of PC and VSV-G was not affected, whereas at an intermediate level (0.5 mg/ml), ER exit of PC, but not of VSV-G, was impaired (Fig. 3C and fig. S10). Higher levels of Sar1H79G (2.5 mg/ml) blocked exit of both cargoes, in agreement with previous reports (13).

A possible mechanism underlying the higher sensitivity of the ER export of PC to Sar1-GTP levels might involve the ability of Sar1-GTP to induce membrane constriction through its N-terminal amphipathic helix (14, 15). We tested our hypothesis by using electron tomography and 3D reconstruction to analyze the organization of ERES in SEDT fibroblasts. Relative to control cells, ERES in SEDT fibroblasts contained a high number of tubular buds that appeared as round profiles connected with each other as beads on a string because of multiple constrictions, too narrow to allow the incorpo-

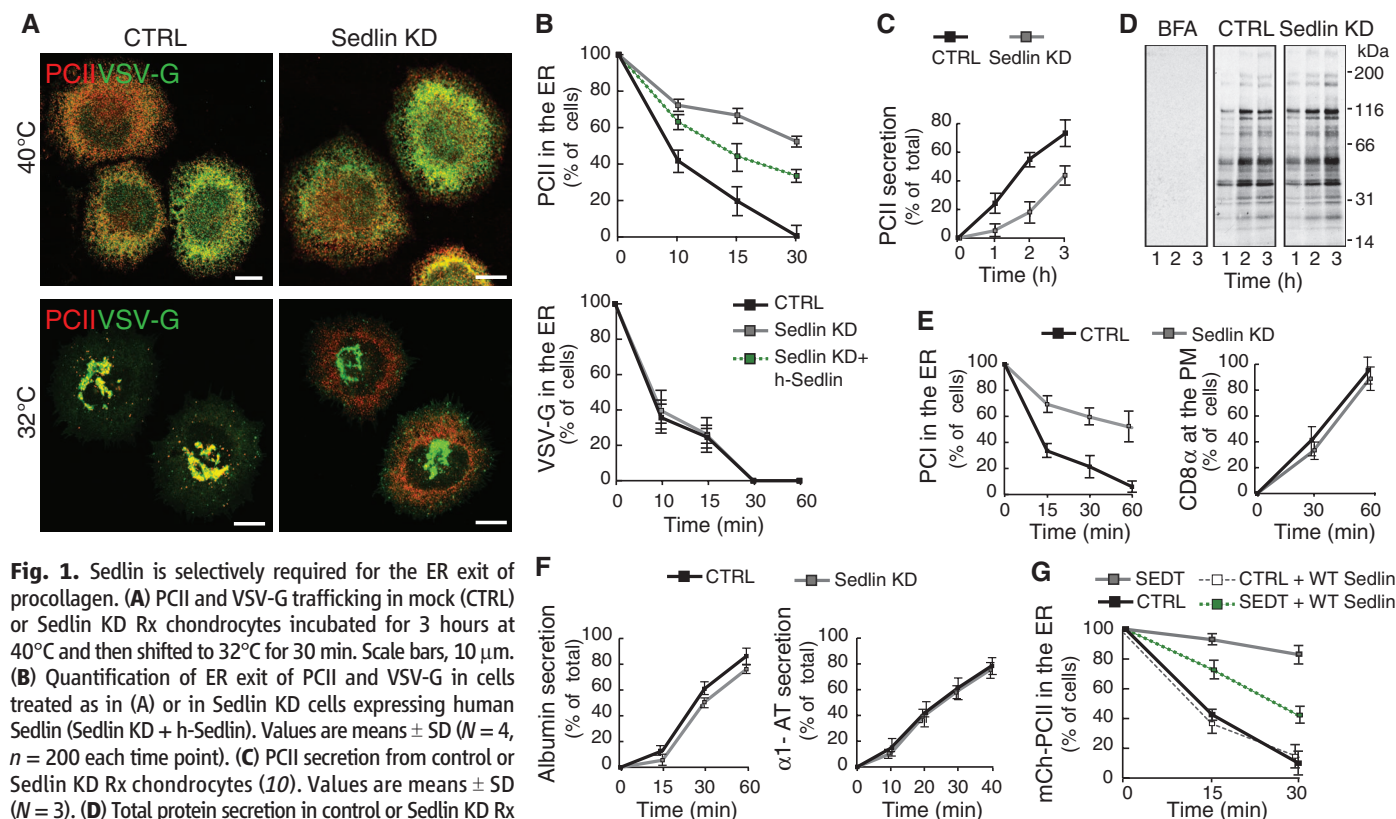


Fig. 1. Sedlin is selectively required for the ER exit of procollagen. (A) PCII and VSV-G trafficking in mock (CTRL) or Sedlin KD Rx chondrocytes incubated for 3 hours at 40°C and then shifted to 32°C for 30 min. Scale bars, 10 μm. (B) Quantification of ER exit of PCII and VSV-G in cells treated as in (A) or in Sedlin KD cells expressing human Sedlin (Sedlin KD + h-Sedlin). Values are means ± SD ($N = 4$, $n = 200$ each time point). (C) PCII secretion from control or Sedlin KD Rx chondrocytes (10). Values are means ± SD ($N = 3$). (D) Total protein secretion in control or Sedlin KD Rx chondrocytes (10). As a negative control, mock cells were treated with brefeldin A (BFA; 10 μg/ml), a blocker of protein secretion. (E) Trafficking of PCII and CD8α in control or Sedlin KD fibroblasts (PM, plasma membrane). Values are means ± SD ($N = 4$, $n = 100$ each time point). (F) Secretion of albumin and α1-antitrypsin (α1-AT) in control and Sedlin KD HepG2 cells

(10). Values are means ± SD ($N = 3$). (G) Quantification of mCherry-PCII ER exit in control or SEDT fibroblasts with or without coexpression of wild-type Sedlin. Cells were incubated for 3 hours at 40°C (time point 0) and then shifted to 32°C for 15 and 30 min. Values are means ± SD ($N = 3$, $n = 100$ each time point).

ration of large cargoes (Fig. 3D). Thus, Sedlin depletion, by inducing an increase in Sar1-GTP that can constrict membranes, appears to lead to the formation of abnormal carriers that are inadequate to incorporate the large PC prefibrils. We finally asked how Sedlin could affect the state of Sar1 and explored the possibility that the two proteins physically interact. Recombinant Sedlin bound both Sar1A and Sar1B (Fig. 4, A and B) preferentially in their GTP state (Fig. 4C). Among the TRAPP subunits tested, only Sedlin bound Sar1 (fig. S11). Because Sar1-GTP also interacts with Sec23 (16), we assessed whether Sedlin and Sec23 coexist in a complex with Sar1. Sedlin pulled down Sec23 only in the presence

of Sar1 (Fig. 4D), indicating that Sedlin does not bind Sec23 directly and that the three proteins instead form a complex. TANGO1 did not affect the formation of the Sedlin–Sar1-GTP–Sec23 complex (Fig. 4D). Taking advantage of the structural homology between Sedlin, a longin-domain protein (17), and SRX, the longin domain of SR α (one of the two SRP receptor subunits) (18) and between Sar1 and SR β (the other SRP receptor subunit belonging to the ARF-Sar GTPase superfamily) (18), we modeled the Sedlin-Sar1 complex on the SRX-SR β complex (18) and identified a putative Sedlin-Sar1 interaction surface (fig. S12). Mutating a residue [His¹³ → Ala

(H13A); fig. S12] in this surface greatly impaired the Sar1-binding ability of Sedlin (Fig. 4E). Moreover, in contrast to wild-type Sedlin, SedlinH13A was not recruited by Sar1H79G in intact cells (Fig. 4F). Furthermore, relative to wild-type Sedlin, disease-associated Sedlin mutants exhibited a significantly lower affinity for Sar1 (Fig. 4G and fig. S11). We have shown that Sedlin, acting in concert with TANGO1, is required for biogenesis of the megacarriers through which PC exits the ER. In our working hypothesis (fig. S13), the combined action of TANGO1 and Sedlin coordinates the stabilization of the inner COPII layer (through TANGO1) with efficient Sar1 cycling (through

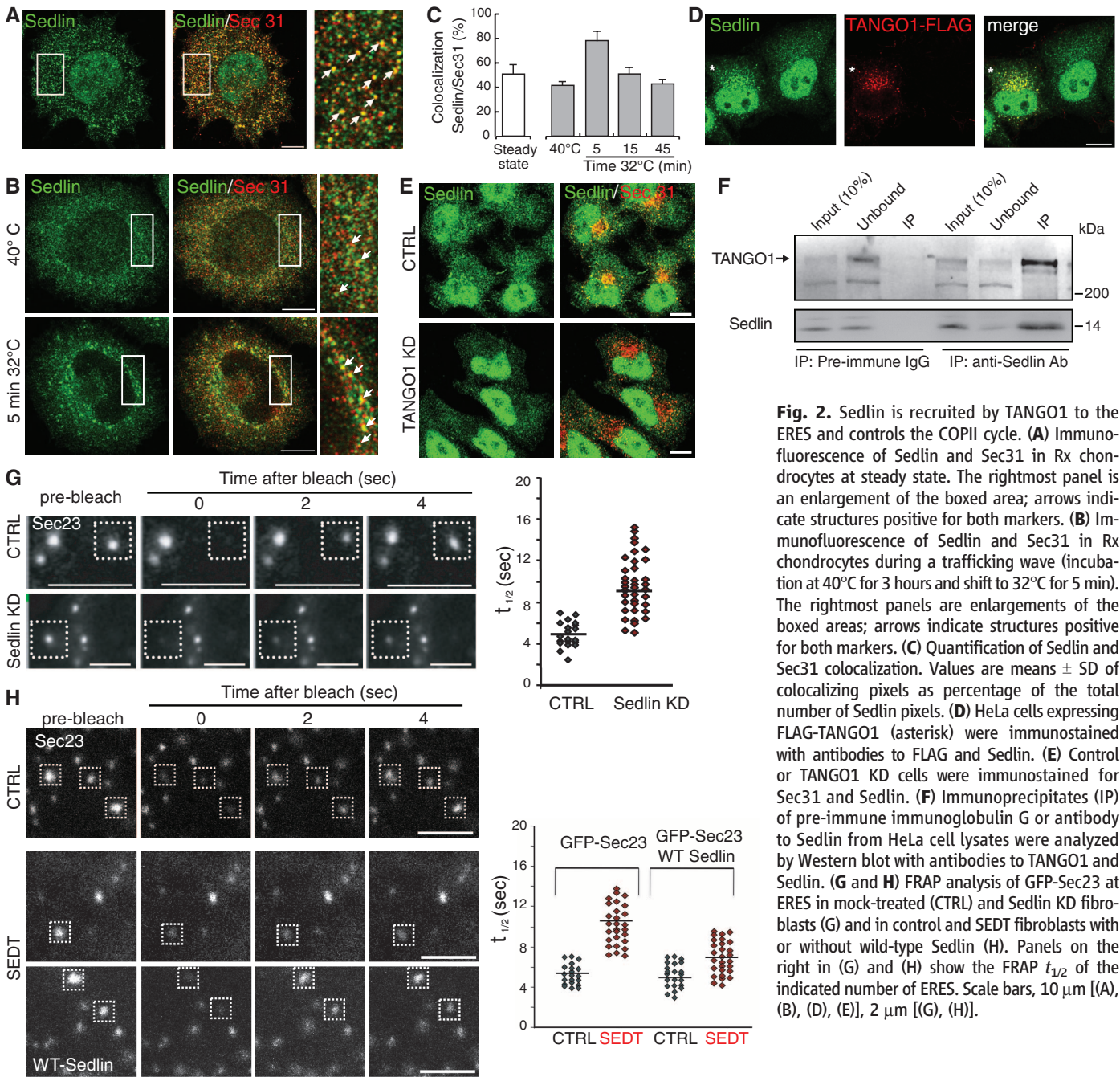


Fig. 2. Sedlin is recruited by TANGO1 to the ERES and controls the COPII cycle. (A) Immunofluorescence of Sedlin and Sec31 in Rx chondrocytes at steady state. The rightmost panel is an enlargement of the boxed area; arrows indicate structures positive for both markers. (B) Immunofluorescence of Sedlin and Sec31 in Rx chondrocytes during a trafficking wave (incubation at 40°C for 3 hours and shift to 32°C for 5 min). The rightmost panels are enlargements of the boxed areas; arrows indicate structures positive for both markers. (C) Quantification of Sedlin and Sec31 colocalization. Values are means $\pm$ SD of colocalizing pixels as percentage of the total number of Sedlin pixels. (D) HeLa cells expressing FLAG-TANGO1 (asterisk) were immunostained with antibodies to FLAG and Sedlin. (E) Control or TANGO1 KD cells were immunostained for Sec31 and Sedlin. (F) Immunoprecipitates (IP) of pre-immune immunoglobulin G or antibody to Sedlin from HeLa cell lysates were analyzed by Western blot with antibodies to TANGO1 and Sedlin. (G and H) FRAP analysis of GFP-Sec23 at ERES in mock-treated (CTRL) and Sedlin KD fibroblasts (G) and in control and SEDT fibroblasts with or without wild-type Sedlin (H). Panels on the right in (G) and (H) show the FRAP $t_{1/2}$ of the indicated number of ERES. Scale bars, 10 μ m [(A), (B), (D), (E)], 2 μ m [(G), (H)].

Fig. 3. Sedlin depletion or mutation increases Sar1-GTP and induces membrane constrictions in nascent carriers at ERES. **(A)** Membrane association of Sar1, Sec23, and Sec31 evaluated as described in (10) in permeabilized control or Sedlin KD HeLa cells in the presence, where indicated, of 100 μ M GTP γ S. Values are means $\pm$ SD ($N = 4$, run in duplicate). **(B)** Control and Sedlin KD cells immunostained with antibodies to Sar1-GTP (10) and Sec31. Numbers below the Merge panels indicate the extent of colocalization of Sec31 with Sar1-GTP. Scale bars, 10 μ m. **(C)** Quantification of the ER exit of PCII and VSV-G in Rx chondrocytes microinjected with Sar1H79G as indicated. Values are means $\pm$ SD ($N = 3$, $n = 200$ each time point). **(D)** Tomograms and 3D reconstruction of ERES in fibroblasts from healthy subjects (CTRL) and SEDT patients. COPII-coated buds (yellow), ER (green), and uncoated tubules (brownish) are highlighted. The incidence of buds with constrictions and constrictions per bud were higher in SEDT (54% and 3 to 6 constrictions per bud) than in controls (15% and 1 or 2 constrictions per bud).

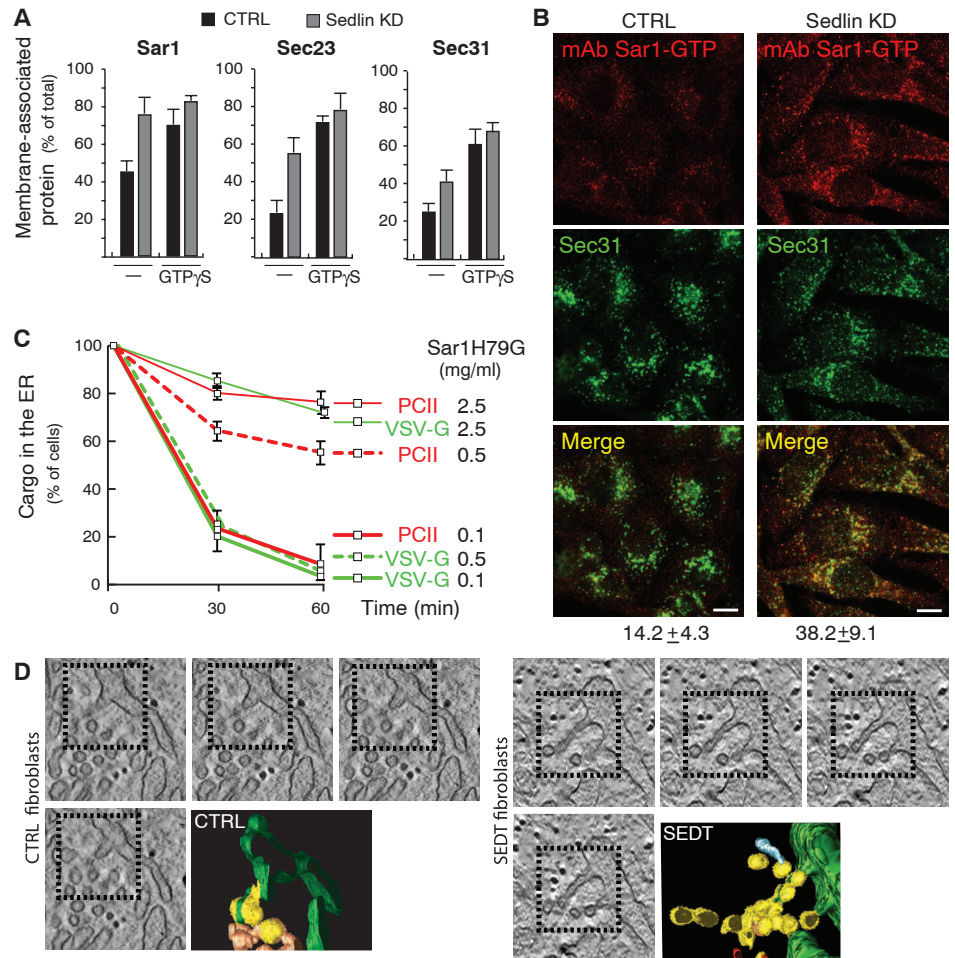
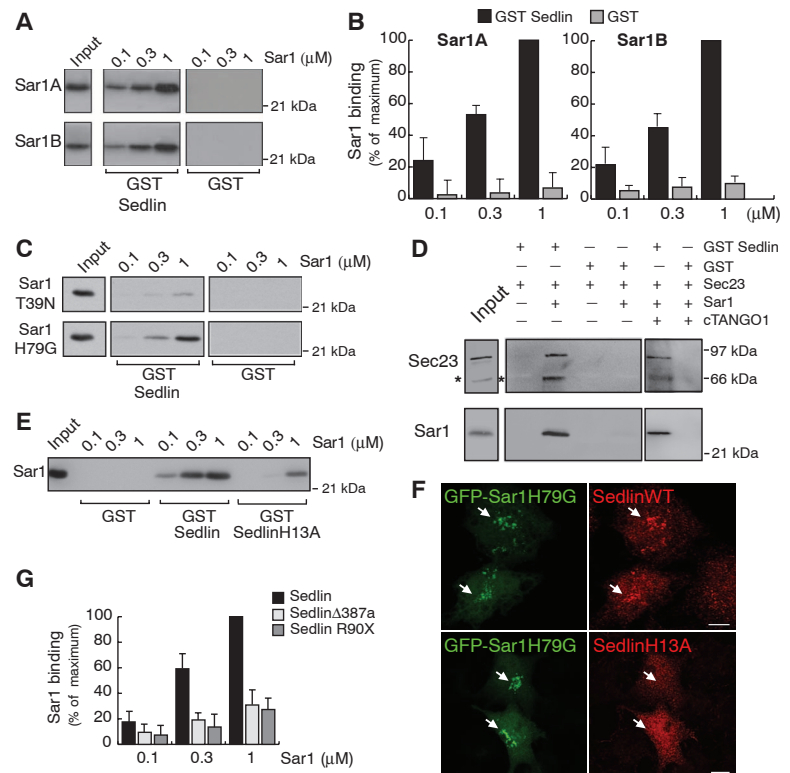


Fig. 4. Sedlin binds Sar1-GTP. **(A)** Pull-down assays of Sar1A and Sar1B with 0.5 μ M GST-Sedlin or GST. Sar1 input, 200 ng. **(B)** Quantification of Sedlin-Sar1 binding expressed as percent binding to 1 μ M His-Sar1 (maximum). Values are means $\pm$ SD ($N = 3$). **(C)** As in (A) using His-tagged GDP- or GTP-restricted Sar1 mutant proteins (Sar1T39N and Sar1H79G, respectively). **(D)** Pull-down assays of GST-Sedlin or GST incubated with Sec23A (0.25 μ M) in the absence or presence of 1 μ M Sar1 and/or the cytosolic domain (amino acids 1211 to 1908) of TANGO1 (cTANGO1). Inputs: 10% of Sec23 and 2.5% of Sar1. Asterisks mark a degradation product of the His-Sec23 protein. **(E)** The same assay as in (A) using His-Sar1B with GST-Sedlin, GST-SedlinH13A, or GST. **(F)** GFP-Sar1H79G was coexpressed in HeLa cells with either wild-type Sedlin or SedlinH13A. Arrows indicate Sar1-positive areas. Scale bars, 10 μ m. **(G)** Quantification of pull-down assays of Sar1 with GST, GST-Sedlin, or GST-tagged disease-associated Sedlin mutants (Sedlin Δ 387a and SedlinR90X), expressed as percentage of GST-Sedlin binding to 1 μ M Sar1 (maximum). Values are means $\pm$ SD ($N = 3$).



Sedlin) to prevent premature membrane constriction, thus allowing growth of nascent carriers and the incorporation of large PC prefibrils.

Our finding that Sedlin binds Sar1 adds a further layer to interactions between TRAPP and COPII, because Bet3 binds Sec23 (19), but only after Sar1 has been released from membranes and vesicles have detached from ER membranes (20). The Sedlin-Sar1 interaction precedes release of COPII vesicles and accelerates dissociation of Sar1 from membranes, thus allowing the next layer of interaction between COPII and TRAPP. TRAPP would then play a role in subsequent events such as Rab1 activation (7), thereby exhibiting the unique property of coupling the cycles of Sar1 and Rab1, two GTPases acting in series in ER-to-Golgi transport.

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Supplementary Materials

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Materials and Methods

Figs. S1 to S13

Table S1

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Radical SAM-Dependent Carbon Insertion into the Nitrogenase M-Cluster

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The active site of nitrogenase, the M-cluster, is a metal-sulfur cluster containing a carbide at its core. Using radiolabeling experiments, we show that this carbide originates from the methyl group of S-adenosylmethionine (SAM) and that it is inserted into the M-cluster by the assembly protein NifB. Our SAM cleavage and deuterium substitution analyses suggest a similarity between the mechanism of carbon insertion by NifB and the proposed mechanism of RNA methylation by the radical SAM enzymes RlmN and Cfr, which involves methyl transfer from one SAM equivalent, followed by hydrogen atom abstraction from the methyl group by a 5'-deoxyadenosyl radical generated from a second SAM equivalent. This work is an initial step toward unraveling the importance of the interstitial carbide and providing insights into the nitrogenase mechanism.

The M-cluster serves as the active site of Mo-nitrogenase, where inert nitrogen is converted to bioavailable ammonia (1). Arguably one of the most complex protein-bound metalloclusters, the M-cluster has a core composition of 1Mo:7Fe:9S and can be viewed as [MoFe₃S₃] and [Fe₄S₃] subclusters bridged by three sulfide atoms (Fig. 1). In addition, an endogenous homocitrate moiety is attached to the Mo and a μ₆-light atom is coordinated at the center of the metal-sulfur core (Fig. 1). The initial discovery of the interstitial atom was exciting because of its potential relevance to the nitrogenase mechanism (2). Recent studies identified this light atom as carbide (3, 4), prompting ques-

tions as to where this carbide originates and how it is inserted into the M-cluster.

The M-cluster is synthesized sequentially on two assembly proteins, NifB and NifEN, before it is delivered to NifDK, the catalytic component of nitrogenase (5). Functional analyses of NifB provided initial insights into the carbon insertion process. NifB has a CXXXXCXXC signature motif that is characteristic of a family of radical S-adenosylmethionine (SAM or AdoMet) enzymes (6, 7). Additionally, it contains sufficient ligands to potentially accommodate the entire complement of iron atoms in the M-cluster (5). These observations led to the proposal that NifB uses a SAM-dependent mechanism to generate the Fe-S core of the M-cluster, which is then processed on NifEN into a mature M-cluster and delivered to its target location in NifDK (5). Acquiring proof for this hypothesis has proven to be challenging. Initial attempts to characterize NifB from *Azotobacter vinelandii* were hampered by the instability of this protein in aqueous solutions.

Recently, mimicking the natural *nifN*(3')-(5')*nifB* fusion in *Clostridium pasteurianum*, this problem was circumvented by fusing the *nifB* and *nifN* genes of *A. vinelandii*, resulting in the expression of a NifEN-B fusion protein consisting of two components that act sequentially in M-cluster biosynthesis (8).

Subsequent metal, activity, ultraviolet/visible (UV/vis), and electron paramagnetic resonance (EPR) analyses established the presence of two transient clusters, designated L- and K-cluster, respectively, on the NifEN-B fusion protein (8, 9). The K-cluster consists of a pair of [Fe₄S₄] clusters, whereas the L-cluster is a [Fe₈S₉] cluster that closely resembles the Fe-S core of the M-cluster (8, 10–12) (Fig. 1). Located near the [Fe₄S₄] cluster at the SAM domain of NifB, the two 4Fe units of K-cluster can be coupled into an 8Fe L-cluster in the presence of SAM (8) (Fig. 1). Subsequently, the L cluster is transferred to and matured on NifEN upon insertion of Mo and homocitrate by NifH, an adenosine triphosphate (ATP)-dependent reductase that also serves as the obligate electron donor for NifDK during catalysis (8, 13–15) (Fig. 1). The resultant M-cluster is then transferred from NifEN to NifDK through direct protein-protein interactions (8, 13, 14) (Fig. 1). The sequential conversion of K- to L- to M-cluster suggests that the K- and L-clusters represent consecutive snapshots of M-cluster biosynthesis. Moreover, the SAM dependence of the conversion of K- to L-cluster points to a synthetic route to bridged metalloclusters that relies on radical chemistry at the SAM domain of NifB. Given the ability of SAM to serve as a carbon donor (16–19), it is possible that the interstitial carbide is derived from SAM and that it is incorporated during the formation of the L-cluster on NifB.

The cleavage pattern of SAM upon interaction with NifEN-B provides evidence that SAM is the source of the interstitial carbide (20). High-performance liquid chromatography (HPLC)

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analysis shows that SAM remains intact in the absence of NifEN-B (Fig. 2A, 1) but is cleaved into two products in the presence of NifEN-B and reductant (Fig. 2A, 2). The two products display HPLC retention times characteristic of *S*-adenosyl-homocysteine (SAH or AdoHcy) and 5'-deoxyadenosine (5'-dAH) (Fig. 2A, 3 and 4).

Liquid chromatography–mass spectrometry (LC-MS) analysis further confirms this identification based on mass-to-charge (m/z) ratios and fragmentation patterns of SAH and 5'-dAH (fig. S1). The same pattern of SAM cleavage was documented for RlmN and Cfr, two radical SAM methyltransferases (16–18). One mechanism proposed for the RlmN- and Cfr-catalyzed reactions (fig. S1) involves transfer of the methyl group from one equivalent of SAM to a polypeptide cysteine residue by an S_N2 mechanism, followed by generation of a 5'-deoxyadenosyl radical (5'-dA•) by the reductive cleavage of a second equivalent of SAM. Subsequently, 5'-dA• abstracts a hydrogen atom from the methylcysteine, generating 5'-dAH and a presumed methylene radical on cysteine that initiates the radical-based methylation of RNA (18, 19, 21).

Deuterium substitution experiments illustrate that the reaction catalyzed by NifEN-B also involves hydrogen atom abstraction from the methyl group of SAM. As observed previously for RlmN and Cfr (10), incubation of NifEN-B with [methyl- d_3]-SAM, where the three hydrogen atoms of the methyl group are labeled with deuterium, results in a mixture of deuterium-enriched 5'-dAD and unlabeled 5'-dAH (Fig. 2B, right). The formation of 5'-dAD reflects the abstraction of deuterium from the labeled methyl group by 5'-dA•, whereas the detection of 5'-dAH suggests the occurrence of (i) abortive cleavage of SAM, in which the 5'-dA• abstracts a solvent-derived hydrogen atom (17, 18); (ii) hydrogen atom abstraction by 5'-dA• from an unlabeled methyl group, which is generated on NifEN-B through the action of unlabeled SAM during cell growth and remains associated with NifEN-B after purification; or (iii) a combination of both events. These experiments firmly establish the initial hydrogen removal from the methyl group as a net transfer of a hydrogen atom. Continued removal of hydrogen atoms from this group, which is required

for the generation of a carbide, likely occurs through proton abstraction via acid/base chemistry (22, 23); alternatively, it could involve the transfer of a hydride to Fe^{3+} and the subsequent removal of protons by the metal cluster in NifEN-B, a mechanism analogous to the conversion of H_2 to H^+ by NiFe hydrogenase (24) and a single Fe model complex (25).

The similarity between NifB- and RlmN/Cfr-catalyzed reactions in using a SAM-derived methyl group as a carbon source raises the possibility of directly visualizing the entry of carbon into NifEN-B and the flow of carbon from NifEN-B onward through carbon-14 (^{14}C) labeling experiments. Such experiments can be carried out by incubating [methyl- ^{14}C]-SAM with (i) NifEN-B alone or (ii) NifEN-B, NifH, and apo NifDK in a maturation assay that also contains dithionite, ATP, molybdate, and homocitrate (13). The former allows the SAM-dependent conversion of K-cluster to L-cluster on NifEN-B, which can be used to assess whether carbon is incorporated in the L-cluster; whereas the latter allows the assembly process to proceed with the maturation of L-cluster to M-cluster by NifH and the transfer of M-cluster to apo NifDK, which can be used to determine whether carbon is transferred together with the cluster species between these proteins (see Fig. 1). Alone, the His-tagged NifEN-B can be captured on immobilized metal affinity chromatography (IMAC) resin after incubation with excess [methyl- ^{14}C]-SAM; in a maturation assay, it can be strategically combined with His- or nontagged NifH and apo NifDK, followed by the capture of His-tagged and nontagged proteins on affinity (IMAC) and anion exchange (DEAE) resin, respectively (Fig. 3A and fig. S3).

Indeed, when His-tagged NifEN-B is captured on IMAC resin after incubation with [methyl- ^{14}C]-SAM, it retains radioactivity after extensive wash with buffer, suggesting a transfer of ^{14}C label from [methyl- ^{14}C]-SAM to NifEN-B (Fig. 3A, 1).

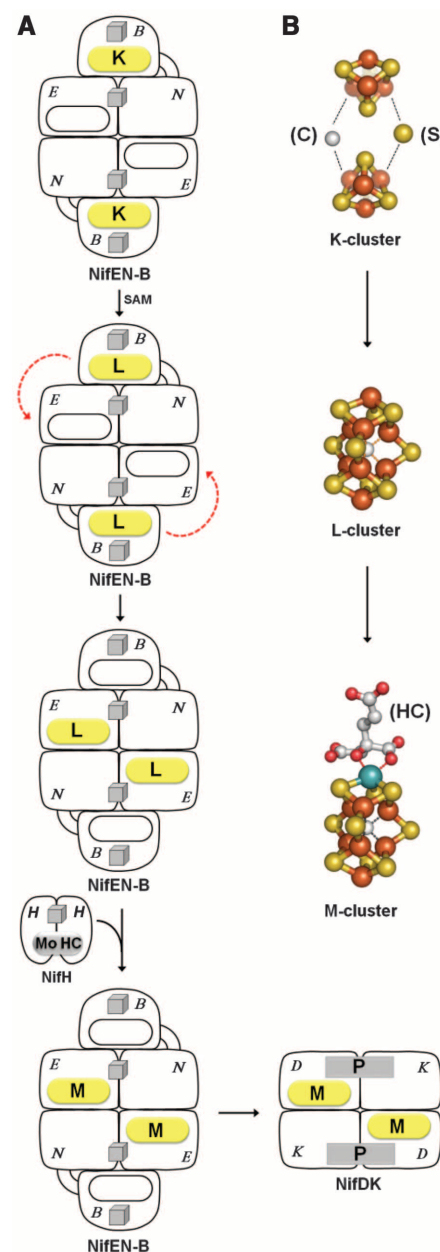
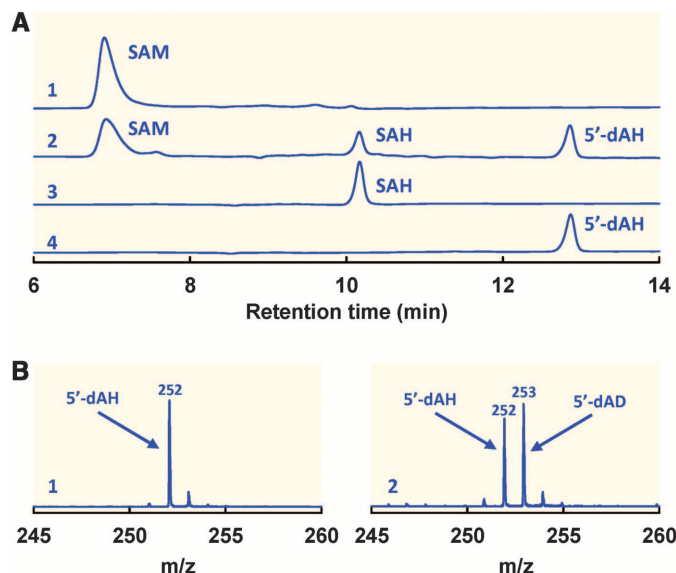


Fig. 1. (A) Biosynthesis of M-cluster on NifEN-B fusion protein, which involves SAM-dependent conversion of K-cluster to L-cluster on NifB, transfer of L-cluster to NifEN, maturation of L-cluster upon NifH-mediated insertion of Mo and homocitrate, and transfer of the resultant M-cluster to NifDK. **(B)** Coupling of the 4Fe units of K-cluster into an 8Fe L-cluster by carbon and sulfur insertion, and conversion of the L-cluster to a mature M-cluster upon insertion of Mo and HC (homocitrate). The L-cluster represents an all-iron homolog of the M-cluster.

Fig. 2. (A) HPLC elution profiles of SAM in the (1) absence and (2) presence of NifEN-B and dithionite, compared with those of (3) SAH and (4) 5'-dAH standards. **(B)** LC-MS analysis of 5'-dAH recovered after incubation with unlabeled SAM (left) or [methyl- d_3]-SAM (right). The ratio of 5'-dAH to SAH produced in the presence of NifEN-B was 1.8 ± 0.1 ($N = 5$ repetitions). In a maturation assay, the yields of SAH and M cluster were 2.0 ± 0.2 and $1.7 \pm 0.1 \mu M$, respectively, in the presence of $10 \mu M$ SAM and $10 \mu M$ NifEN-B ($N = 5$ repetitions).



After the incubation of His-tagged NifEN-B with nontagged NifH and His-tagged NifDK in a maturation assay, the ^{14}C label can be detected in the IMAC fraction (i.e., NifEN-B and NifDK) but not in the DEAE fraction (i.e., NifH), indicating that the ^{14}C label is not harbored on NifH (Fig. 3A, 2). When His-tagged NifDK is replaced by nontagged NifDK in the same maturation assay, however, the ^{14}C label appears in the DEAE fraction (i.e., NifH and NifDK), suggesting a transfer of ^{14}C label from NifEN-B to NifDK (Fig. 3A, 3). A further substitution of His-tagged NifH for nontagged NifH in this assay clearly shows the presence of radioactivity in the DEAE fraction (i.e., NifDK), providing definitive proof for the transfer of ^{14}C label to NifDK upon cluster maturation (Fig. 3A, 4). When [methyl- ^{14}C]-SAM is replaced by [carboxyl- ^{14}C]-SAM in the same set of experiments, radioactivity is not detected in any of the protein fractions (Fig. 3B). Thus, the radioactive labeling of NifEN-B and

NifDK does not originate from the carboxyl group or from the nonspecific binding of SAM to these proteins; rather, it originates from the ^{14}C -labeled methyl group of SAM.

The ^{14}C label can be further traced to the cluster species extracted from NifEN-B and NifDK. The amount of extractable ^{14}C -labeled cluster species on NifEN-B (Fig. 3C, 1, left) is substantially decreased after the maturation of L-cluster and transfer of M-cluster from NifEN-B to apo NifDK (Fig. 3C, 2, left), and such a decrease is accompanied by an appreciable accumulation of extractable ^{14}C -labeled cluster species on NifDK after the incorporation of M-cluster (Fig. 3C, 2, middle). Activity analyses confirm that the clusters extracted from NifEN-B and NifDK are L- and M-cluster, respectively, because the former needs to be matured further on NifH before it can be inserted into apo NifDK (Fig. 3D, 4 and 5), whereas the latter can be used directly to reconstitute apo NifDK (Fig. 3D, 6). SDS-

polyacrylamide gel electrophoresis analysis further demonstrates the strict association of ^{14}C label with the cluster species, because no radioactivity is detected in the polypeptides of [methyl- ^{14}C]-SAM-treated NifEN-B or NifDK (fig. S4). The absence of ^{14}C label from NifEN-B polypeptides also implies a direct transfer of methyl-derived carbon species to the K-cluster. This is supported by data from a posttranslational modification (PTM) analysis, which reveal the absence of methyl or methylene group from NifEN-B polypeptides after treatment with SAM (26).

Based on these observations, we propose two plausible mechanisms for the carbon insertion by NifB, which are clearly distinct in the requirement for reductant in the initial step of methyl transfer (Fig. 4). The first mechanism (Fig. 4A) begins with the transfer of a methyl group from SAM to a sulfide of the K-cluster through a reductant-independent $\text{S}_\text{N}2$ mechanism, generating SAH and a cluster that at least transiently

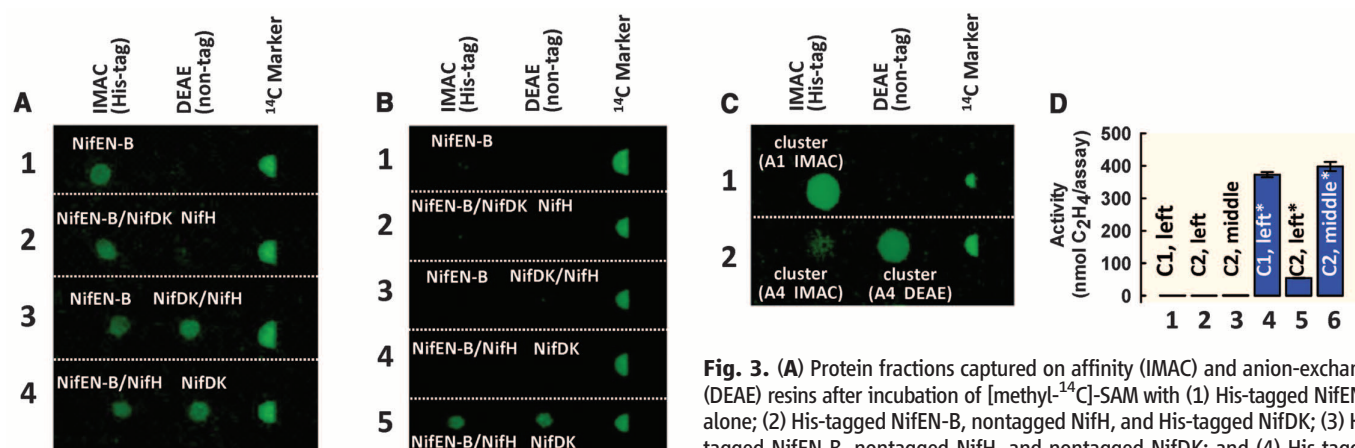
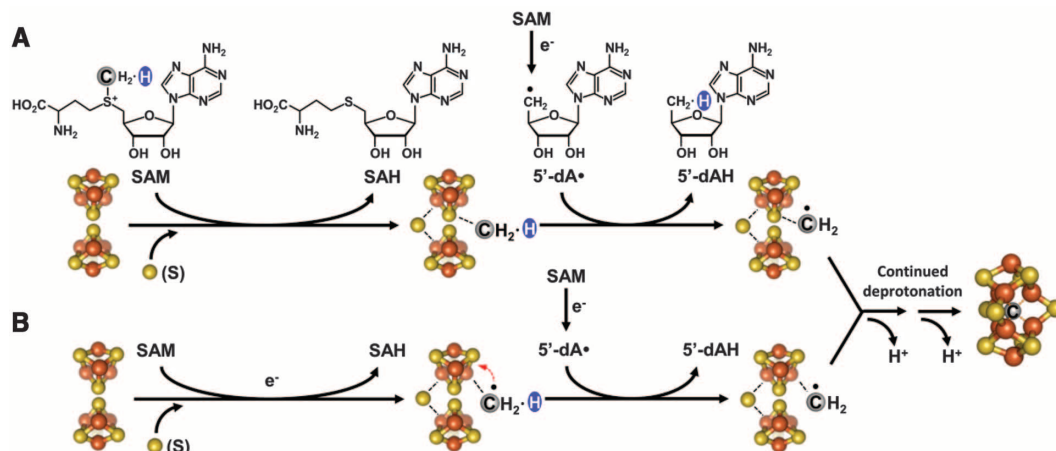


Fig. 3. (A) Protein fractions captured on affinity (IMAC) and anion-exchange (DEAE) resins after incubation of [methyl- ^{14}C]-SAM with (1) His-tagged NifEN-B alone; (2) His-tagged NifEN-B, nontagged NifH, and His-tagged NifDK; (3) His-tagged NifEN-B, nontagged NifH, and nontagged NifDK; and (4) His-tagged NifEN-B, His-tagged NifH, and nontagged NifDK. Assays 2 to 4 also contained

dithionite, ATP, molybdate, and homocitrate. (B) Experiments (B, 1 to 4) were identical to those in (A, 1 to 4), except that [carboxyl- ^{14}C]-SAM was used instead of [methyl- ^{14}C]-SAM. Experiment (A, 4) was included as a positive control here (B, 5). (C) Clusters extracted from (1) the IMAC fraction in (A, 1); and (2) the IMAC fraction (left) and DEAE (middle) fraction in (A, 4). (D) Activities of C_2H_4 reduction by extracted clusters alone (1 to 3), after maturation and transfer of clusters to apo NifDK (4 and 5), or upon direct transfer of cluster to apo NifDK (6). Clusters used for activity analyses were from C1, left (1 and 4); C2, left (2 and 5); and C2, middle (3 and 6), respectively. Activities of clusters upon maturation and/or incorporation into apo NifDK (4-6) are indicated by *.

Fig. 4. Proposed mechanisms of carbide insertion involve (A) transfer of a methyl group from SAM to the 4Fe K-cluster through an $\text{S}_\text{N}2$ mechanism, followed by reductive cleavage of a second equivalent of SAM to generate 5-dA $^\bullet$, which abstracts a hydrogen atom from the methyl group on the K-cluster; or (B) reductive cleavage of a methyl group from SAM, followed by transfer of the resultant methyl radical to the 4Fe K-cluster and abstraction of a hydrogen atom of this group by 5-dA $^\bullet$. Continued deprotonation of the C intermediate in (A) or (B) must occur concomitant with its incorporation into the 8Fe L-cluster as an interstitial carbide atom. Atoms of the clusters are colored as follows: Fe, orange; S, yellow; C, gray.



contains a methanethiol ligand. Then, reductive cleavage of a second equivalent of SAM gives rise to a 5'-dA•, which abstracts a hydrogen atom from the methyl group, generating 5'-dAH and a methylene thiol radical. This scenario is consistent with the fact that Fe²⁺ in the K-cluster is not sufficiently nucleophilic for the initial methyl group transfer to generate an Fe-C bond (27). Consequently, it must involve a switch of the ligation of the C intermediate to Fe atom(s), as well as the removal of the other two hydrogen atoms of this intermediate, which eventually leads to the formation of an interstitial carbide atom that is coordinated by six Fe atoms. The second mechanism (Fig. 4B) starts with the reductive cleavage of a methyl group from SAM, which generates a transient methyl radical that could be captured on Fe²⁺ of the K-cluster to directly form an Fe-C bond. This scenario is analogous to the capture of C radicals by Co²⁺ in vitamin B12 radical chemistry (28, 29), and it eliminates the need for ligand exchange of the C intermediate. The subsequent hydrogen transfer could proceed in a manner identical to that in the first mechanism, which involves the initial hydrogen atom abstraction by 5'-dA• and the subsequent proton abstraction that leads to the formation of carbide.

It should be noted that the radical SAM-based carbide insertion is an integral part of a synthetic strategy to couple the two 4Fe units of K-cluster into an 8Fe L-cluster (Fig. 4). It is accompanied by the incorporation of a cluster-bridging sulfide, as well as the structural rearrangements of Fe-S clusters through the facile ligand replacement of their tetrahedral Fe²⁺/Fe³⁺ atoms by an addition/elimination mechanism (30, 31). Details of this

process, such as whether the initial methyl transfer requires a reductant and how the starting methyl group is processed into a carbide atom, are yet to be defined. Nevertheless, this work is a step toward unraveling the structure-function relationship of the interstitial carbide, which could provide insights into the mechanism of nitrogenase.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6102/1672/DC1
Materials and Methods
Figs. S1 to S4
References (32–36)

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Evidence of Abundant Purifying Selection in Humans for Recently Acquired Regulatory Functions

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Although only 5% of the human genome is conserved across mammals, a substantially larger portion is biochemically active, raising the question of whether the additional elements evolve neutrally or confer a lineage-specific fitness advantage. To address this question, we integrate human variation information from the 1000 Genomes Project and activity data from the ENCODE Project. A broad range of transcribed and regulatory nonconserved elements show decreased human diversity, suggesting lineage-specific purifying selection. Conversely, conserved elements lacking activity show increased human diversity, suggesting that some recently became nonfunctional. Regulatory elements under human constraint in nonconserved regions were found near color vision and nerve-growth genes, consistent with purifying selection for recently evolved functions. Our results suggest continued turnover in regulatory regions, with at least an additional 4% of the human genome subject to lineage-specific constraint.

Initial sequencing of the human genome revealed that 98.5% of human DNA does not code for protein (1), raising the question of what fraction of the remaining genome is func-

tional. Mammalian conservation suggests that ~5% of the human genome (2, 3) is conserved due to noncoding and regulatory roles, but more than 80% is transcribed, bound by a regulator, or

associated with chromatin states suggestive of regulatory functions (4–6). This discrepancy may result from nonconsequential biochemical activity or lineage-specific constraint (7, 8). Similarly, evolutionary turnover in regulatory regions (9–11) may be due to nonconsequential activity in neutrally evolving regions in each species or turnover in functional elements associated with turnover in activity. To resolve these questions, we need new methods for measuring constraint within a species, rather than between species.

Single-nucleotide polymorphisms (SNPs) within human populations have been identified only every 153 bases on average (12), compared with 4.5 substitutions per site among the genomes of 29 mammals (2), making it impossible to detect individual constrained elements (13). Instead, aggregate measures of human diversity across thousands of dispersed elements are needed. Such

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measures have been used to show that human constraint correlates with mammalian conservation (4, 14–17), mRNA splice sites (18), and regulatory elements (19), and that similar selective pressures act in humans and across mammals (2). However, differences between mammalian and human constraint remain unresolved. Recent positive selection has been detected by unexpectedly many recent substitutions (20) or extreme pat-

Fig. 1. (A) Only a small fraction (purple) of biochemically active regions (red) overlaps conserved elements (blue). (B) Active regions (red) show reduced heterozygosity relative to inactive regions outside conserved elements (white), suggesting lineage-specific purifying selection (black arrow). Conserved elements that lack activity (blue) show increased human heterozygosity relative to active conserved regions (purple), suggesting recent loss of selective constraint (white arrow). (C and D) Comparison of mean heterozygosity for ENCODE-annotated elements (red) versus non-ENCODE elements (black) and active chromatin (green) versus inactive (blue) shows a consistent reduction at varying genetic distances from exons (C) and varying expected background selection (D), confirming that the heterozygosity reduction is due to purifying selection. Shaded regions represent a 95% confidence interval on the mean heterozygosity if one assumes independence between bases.

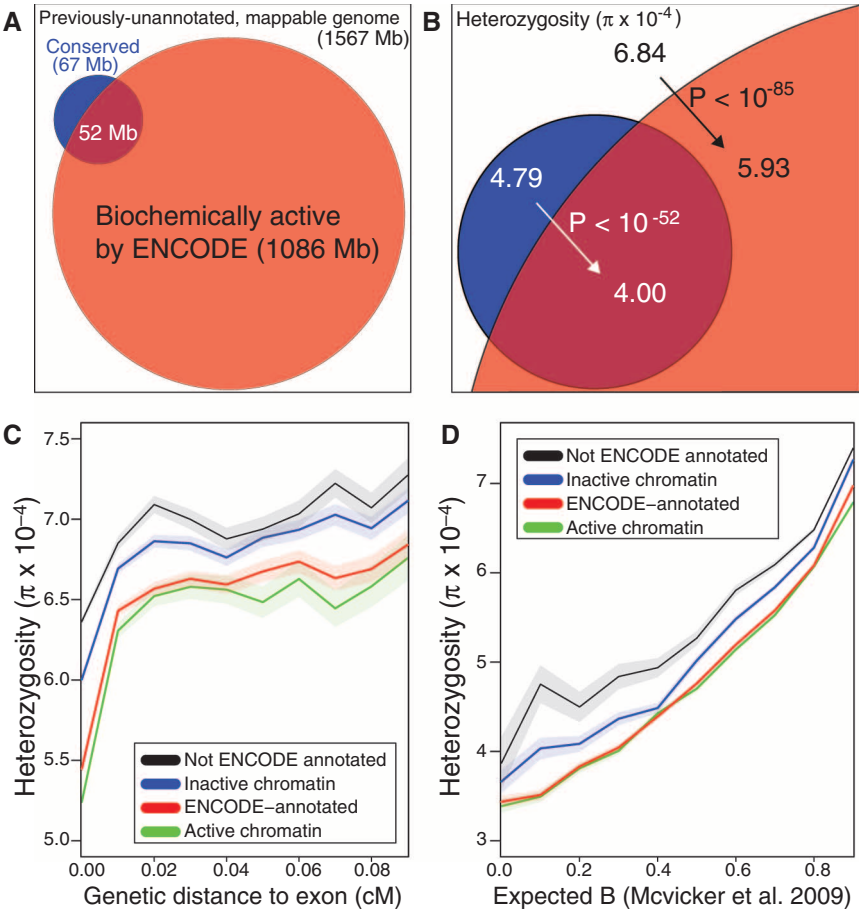
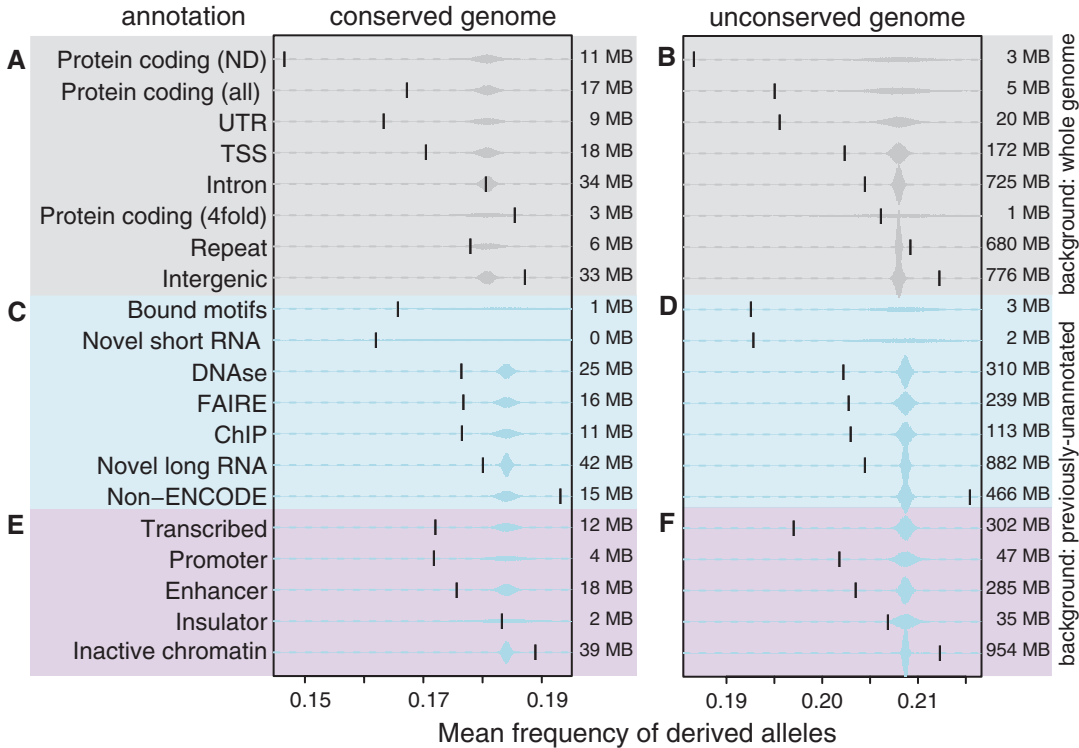


Fig. 2. Each row shows the mean frequency of derived alleles (vertical bar) found within the specified annotation type, relative to genomic samples (histogram) from the specified background (right column). These are shown for previously annotated features (A and B), ENCODE-defined features (C and D), and chromatin states (E and F), in both conserved regions [(A), (C), and (E)] and nonconserved regions [(B), (D), and (F)]. Region sizes are specified.



terms of linkage disequilibrium (LD) and population differentiation (21). However, recent negative selection has not been investigated, as the paucity of variants segregating in the global population makes a selective decrease in the diversity of any given locus indistinguishable from a fortuitous one.

Combining population genomic information from the 1000 Genomes Project (12) and biochemical data of the Encyclopedia of DNA Elements (ENCODE) Project (5), we estimated constraint associated with diverse genomic functions in aggregate over 1567 Mb of “previously unannotated” regions encompassing 4.7 million SNPs, excluding exons, proximal promoter regions, and artifact-prone regions (22) (Fig. 1A).

On the basis of SNP density, heterozygosity, and derived allele frequency (DAF), we developed a statistical procedure for measuring genome-wide constraint accounting for mutation rate biases and interdependence of allele frequencies due to LD (22). All P values are derived from this test unless otherwise noted. To distinguish whether the increased human constraint in active regions (5) could be due solely to mammalian conservation (Fig. 1B and fig. S1), rather than lineage-specific constraint, we specifically studied regions not conserved across mammals.

Remarkably, nonconserved active regions showed significant evidence of purifying selection: SNP density was 10% lower ($P < 10^{-64}$), heterozygosity 13% ($P < 10^{-85}$), and DAF 5%

($P < 10^{-65}$), compared with reductions of 28%, 33%, and 16%, respectively, for conserved regions. Because nonconserved regions cover a >10-fold larger fraction of the genome, this suggests that a substantial fraction of human constraint lies outside mammalian-conserved regions. The observed decrease in diversity is not due to undetected conserved regions or the threshold used to defined conserved elements (fig. S2), or to background selection (23) (Fig. 1, C and D), biased gene conversion (table S1), or decreased mapping to nonreference alleles (22) (table S2).

The level of human-specific constraint varies with the observed biochemical activity (Fig. 2, figs. S3 to S5, and tables S3 and S4). Short non-coding RNAs are as strongly constrained as protein-coding regions. Long noncoding RNAs (lncRNAs) are significantly constrained in humans, even though they lack significant mammalian conservation (5), suggesting primarily lineage-specific functions. These results are not explained by local mutation rate variation or transcription-mediated repair, as DAF is robust to both.

We also found human-specific constraint across nonconserved regulatory features (Fig. 2, C and D). Regulatory motifs bound by their regulators show constraint similar to coding regions, and consistently higher than for nonbound instances ($P = 9.5 \times 10^{-7}$, binomial test) (Fig. 3). Regulatory regions defined by different assays, including deoxyribonuclease hypersensitivity and transcription factor binding, show significant and similar levels of human constraint. Different chromatin states (5, 24) show levels of constraint according to their roles (Fig. 2, E and F), with promoter states similar to previously annotated transcription start site-proximal regions, enhancer states significant but weaker, and insulators similar to background regions, consistent with enhancer and promoter regions requiring a larger number of motifs than insulator regions. In contrast, regions that do not overlap with active ENCODE elements and inactive chromatin states show even lower constraint than ancestral repeats (Fig. 2, B, D, and F), suggesting that they may provide a more accurate neutral reference than repeats that can have exapted functions (25).

Comparison with primate constraint suggests evolutionary turnover. Mammalian-conserved regions lacking ENCODE activity show reduced human constraint relative to active regions (SNP density $P < 10^{-41}$, heterozygosity $P < 10^{-52}$, DAF $P < 10^{-14}$) (Fig. 1B and fig. S1), suggesting recent loss in function and activity. These also show higher primate divergence relative to active regions, suggesting that some loss of constraint likely predates human-macaque divergence. Conversely, a fraction of lineage-specific elements likely arose in the common ancestor of primates, as human-macaque divergence mirrors human diversity for both active and inactive nonconserved regions (fig. S6).

To gain insights into the functional adaptations likely involved in this turnover, we applied our aggregation approach to regulatory regions

Fig. 3. Average heterozygosity for bound regulatory motif instances (x axis) and nonbound regulatory motif instances (y axis), evaluated in nonconserved regions of the genome to estimate lineage-specific constraint. Shown are all transcription factors with at least 30 kb of bound instances (red points). Numbers in parentheses indicate number of bound and number of nonbound instances, respectively.

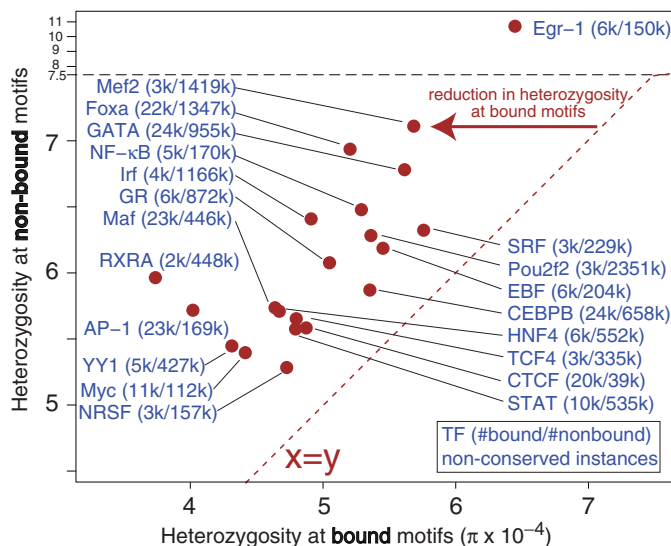
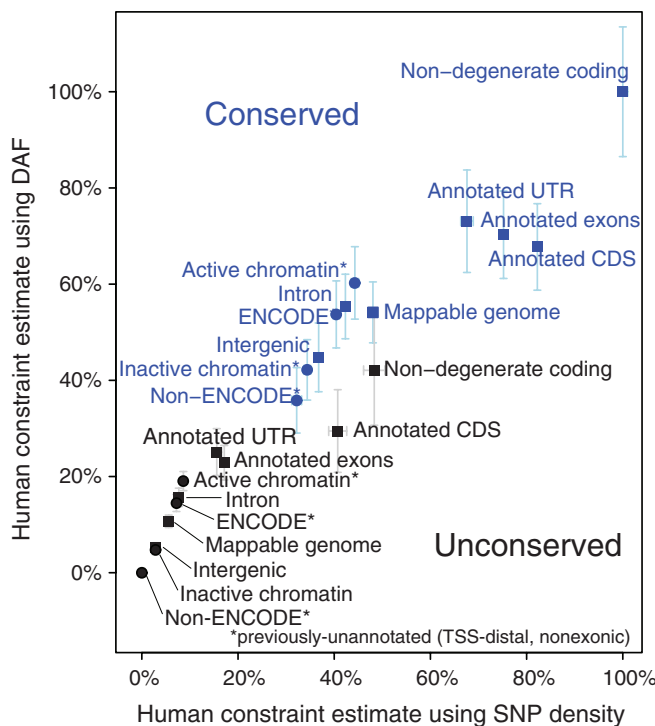


Fig. 4. Estimated PUC in human using SNP density (x axis) and DAF (y axis), across previously annotated elements (squares) and newly annotated ENCODE elements (circles), in both conserved (blue) and nonconserved (black) regions. Error bars denote 95% confidence intervals on the estimates. Each metric was linearly scaled between 0% for non-ENCODE nonconserved regions and 100% for conserved nondegenerate coding positions in each background selection bin separately (fig. S8).



associated with genes of different functions (22). We found that highly constrained nonconserved enhancers are associated with retinal cone cell development [$P < 10^{-4}$ in gene ontology (GO)] and nerve growth ($P < 10^{-5}$ in GO, Reactome, and the Kyoto Encyclopedia of Genes and Genomes) (fig. S7). This evidence of recent purifying selection for regulation of the nervous system and color vision is intriguing given their accelerated evolution in primates (20, 26, 27).

We next studied how the number of aggregated regions affects the ability to discriminate functional elements based on their increased human constraint (fig. S8). We found no discriminative power for individual elements, despite a significant global reduction in heterozygosity ($P < 10^{-20}$, Mann-Whitney-Wilcoxon test on heterozygosity of individual elements), but discriminative power increased significantly as the sample size grew (22).

We estimated the proportion of the human genome under constraint (PUC) after correcting for background selection (fig. S9) and found remarkable agreement between our orthogonal metrics (Fig. 4A). We estimate that an additional 137 Mb (4%) of the human genome is under lineage-specific purifying selection (table S6), consistent with a recent cross-species extrapolation (28).

Our results suggest that almost half of human constraint lies outside mammalian-conserved regions, even though the strength of human constraint is higher in conserved elements. Protein-coding constraint occurs primarily in conserved regions, whereas regulatory constraint is primarily lineage-specific (fig. S10), as proposed during mammalian radiation (29). Although differences in activity between mammals (10, 11) can be interpreted as lack of functional constraint (30), our results suggest instead that turnover in activity is accompanied by turnover in selective constraint. A minority of new regulatory elements lies in recently acquired primate-specific regions (5), but the bulk lies in mammalian-aligned regions that provided raw materials for regulatory innovation.

Genome-wide association studies suggest that 85% of disease-associated variants are noncoding (8), a fraction similar to the proportion of human constraint that we estimate lies outside protein-coding regions (table S6). This suggests that mutations outside conserved elements play important roles in both human evolution and disease and that large-scale experimental assays in multiple individuals, cell types, and populations can provide a means to their systematic discovery.

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Supplementary Materials

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Materials and Methods
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An Immunosurveillance Mechanism Controls Cancer Cell Ploidy

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Cancer cells accommodate multiple genetic and epigenetic alterations that initially activate intrinsic (cell-autonomous) and extrinsic (immune-mediated) oncosuppressive mechanisms. Only once these barriers to oncogenesis have been overcome can malignant growth proceed unrestrained. Tetraploidization can contribute to oncogenesis because hyperploid cells are genomically unstable. We report that hyperploid cancer cells become immunogenic because of a constitutive endoplasmic reticulum stress response resulting in the aberrant cell surface exposure of calreticulin. Hyperploid, calreticulin-exposing cancer cells readily proliferated in immunodeficient mice and conserved their increased DNA content. In contrast, hyperploid cells injected into immunocompetent mice generated tumors only after a delay, and such tumors exhibited reduced DNA content, endoplasmic reticulum stress, and calreticulin exposure. Our results unveil an immunosurveillance system that imposes immunoselection against hyperploidy in carcinogen- and oncogene-induced cancers.

“Oncogenic stress,” generally resulting from the activation of oncogenes or inactivation of oncosuppressor

genes, can ignite an array of intrinsic programs for tumor suppression, some of which are linked to the DNA damage response (1). For instance,

oncogenic stress and DNA damage can induce senescence or apoptotic cell death (1, 2), two cell-intrinsic mechanisms for the suppression of cancer cells, or stimulate the expression of ligands for NKG2D receptors (3) or CD95/FAS (4), which can increase the susceptibility of tumor cells to killing by immune effectors (3–5), elicit an immune response against tumor antigens (6, 7), or both. One prominent mechanism of tumorigenesis involves an initial event of tetraploidization. This can result from illicit cell-to-cell fusion among somatic cells (8), endomitosis (during which cells with duplicated chromosomes fail to divide) (9, 10), or endoreplication (during which cells enter two consecutive rounds of DNA replication that are not separated by mitosis) (11). Tetraploidy has been observed in the early stages of esophageal, colorectal, mammary, and cervical cancers (12). Tetraploidy may act as a stochastic generator of genomic instability because tetraploid cells can gain or lose chromosomes progressively during aberrant rounds of bipolar mitosis or undergo multipolar mitosis (10, 12–15). This oncogenic polyploidization-depolyploidization cascade is normally avoided by cell-autonomous, oncosuppressive control mechanisms (9, 12, 15–19). We report the unexpected finding that the chromosomal content is also controlled indirectly, by an immunosurveillance mechanism that ensures the elimination of hyperploid cells.

Cytotoxic anticancer agents only induce immunogenic cancer cell death if they provoke the

translocation of calreticulin (CRT) to the plasma membrane surface as a consequence of endoplasmic reticulum (ER) stress (6, 20). CRT facilitates the phagocytosis of stressed and dying cells by macrophages as well as by antigen-presenting dendritic cells (21–23). Among 480 compounds that perturb signal transduction pathways, we identified 12 agents that caused a CRT–green fluorescent protein (GFP) fusion protein transduced into human osteosarcoma U2OS cells to accumulate in granules and to move to the cellular periphery (24). These agents included calyculin A, a protein phosphatase-1 inhibitor that stimulates CRT exposure (6), as well as four agents that can induce polyploidization, namely one cytokinesis inhibitor and three inhibitors of microtubule function (Fig. 1A and fig. S1, A to D). The redistribution of endogenous CRT to the surface of the plasma membrane was confirmed by three distinct methods (figs. S1, E to J, and S2). CRT exposure was induced by multiple hyperploidy-promoting conditions in immortalized nontransformed or malignant murine and human cell lines, as well as in *Tp53*^{−/−} primary mouse mammary gland cells, intestinal epithelial cells, and colon crypt organoids, all of which are susceptible to pharmacologically induced or spontaneous polyploidization (9) (figs. S3 and S4, A to D). Human colon carcinoma DLD-1+7 cells, which are trisomic owing to the introduction of one additional copy of chromosome 7 (25), did not display a constitutive ER stress response or CRT exposure yet did expose CRT in response to hyperploidy with cytochalasin D (fig. S4, E and F). Hence, a major, nonphysiological increase in chromosome content stimulates CRT exposure. Similarly to mitoxantrone-induced CRT externalization (20, 26), the microtubular poison-induced exposure of CRT at the cell surface was accompanied by all hallmarks of the ER stress response, namely the PKR (protein kinase, RNA-activated)–like ER kinase (PERK)–associated phosphorylation of eukaryotic initiation factor 2 α (eIF2 α), the up-regulation of X-box binding protein 1 (XBP1), and the perinuclear translocation of activating transcription factor 6 (ATF6) (Fig. 1B and fig. S5). Moreover, the depletion of PERK, caspase-8, or ERp57 (an ER chaperone that is required for CRT exposure); the knockout of *Bax* and *Bak* or *Casp8*; or a nonphosphorylatable mutant of eIF2 α (S51A) all reduced CRT transport to the surface (fig. S6), thus revealing a CRT exposure pathway that was similarly induced by mitoxantrone and microtubular poisons. Mouse colon cancer CT26 cells succumbing in response to the microtubular inhibitor nocodazole protected immunocompetent BALB/c mice against rechallenge with live CT26 cells. The immunogenicity of CT26 cells treated with microtubule inhibitors was strongly reduced by depletion of CRT or ERp57, yet could be restored by the absorption of recombinant CRT to the cells (Fig. 1C and fig. S7).

The surface exposure of CRT and ERp57 was increased in CT26 clones derived from cells transiently exposed to nocodazole that contained close

to twice the DNA content of parental cells (which we refer to as “hyperploid” cells), although the surface expression of most other membrane proteins was unaltered (Fig. 1D and fig. S8). This hyperploidy-associated increase in CRT exposure was also observed in mouse Lewis lung carcinoma (LLC) and fibrosarcoma MCA205 cells, as well as in human cancer cell lines (fig. S9). As compared to their parental counterparts, hyperploid clones exhibited constitutive PERK and eIF2 α phosphorylation (Fig. 1E). Interruption of the CRT exposure pathway reduced the clonogenic potential of hyperploid cells (Fig. 1, F and G), suggesting a functional link between the ER stress–associated CRT exposure pathway and the fitness of hyperploid cells.

Because hyperploidy is linked to CRT exposure, we wondered whether cancer cells with increased DNA content might be subjected to immunosurveillance. We injected parental or hyperploid clones derived from CT26 [class I major histocompatibility complex (MHC) haplotype H2^d], MCA205 (H2^b), or LLC (H2^b) cells subcutaneously into syngeneic immunocompetent wild-type (WT) or immunodeficient *Rag2*^{−/−} γ_c ^{−/−} (Rag γ) mice. When inoculated into immunocompetent (but not immunodeficient) mice, hyperploid CT26 cells formed tumors less frequently than did their parental precursors, and such cancers grew less efficiently (Fig. 1H). Similarly, tumors arising from hyperploid LLC or MCA205 cells generally failed to grow or grew more slowly in C57Bl/6 mice, unless the immune system was compromised by the Rag γ phenotype (fig. S10, A and B). This difference in the growth of cancer cells on immunodeficient versus immunocompetent mice was lost upon depletion of CRT from hyperploid cells (Fig. 1I). Mice that did not develop tumors within 1 month after injection of hyperploid CRT-exposing cancer cells (40 to 50% of all mice) also failed to develop cancers after another inoculation with parental CT26 cells (Fig. 1J) yet allowed for the growth of unrelated cancer cells (fig. S11). The incidence and proliferation rate of tumors generated from hyperploid CT26 cells increased upon the depletion of CD4⁺ or CD8⁺ T lymphocytes from BALB/c mice (Fig. 1K) or when interferon γ (IFN- γ) or the type I interferon receptor 1 (*Ifnar1*) were inactivated (Fig. 1, L and M). Hyperploid cells were more efficient at priming T lymphocytes against tumor antigens than were their parental counterparts, as indicated by experiments in which tumor cells were injected into the footpad, followed by recovery of T lymphocytes from the draining lymph node, their restimulation with tumor antigens, and quantification of IFN γ production. CRT depletion from hyperploid tumor cells reduced T lymphocyte priming in this system (fig. S10C).

Histological examination of tumors arising from hyperploid CT26 clones in either immunodeficient Rag γ or immunocompetent BALB/c mice revealed a difference in the mean nuclear diameter, which was decreased in tumors from BALB/c

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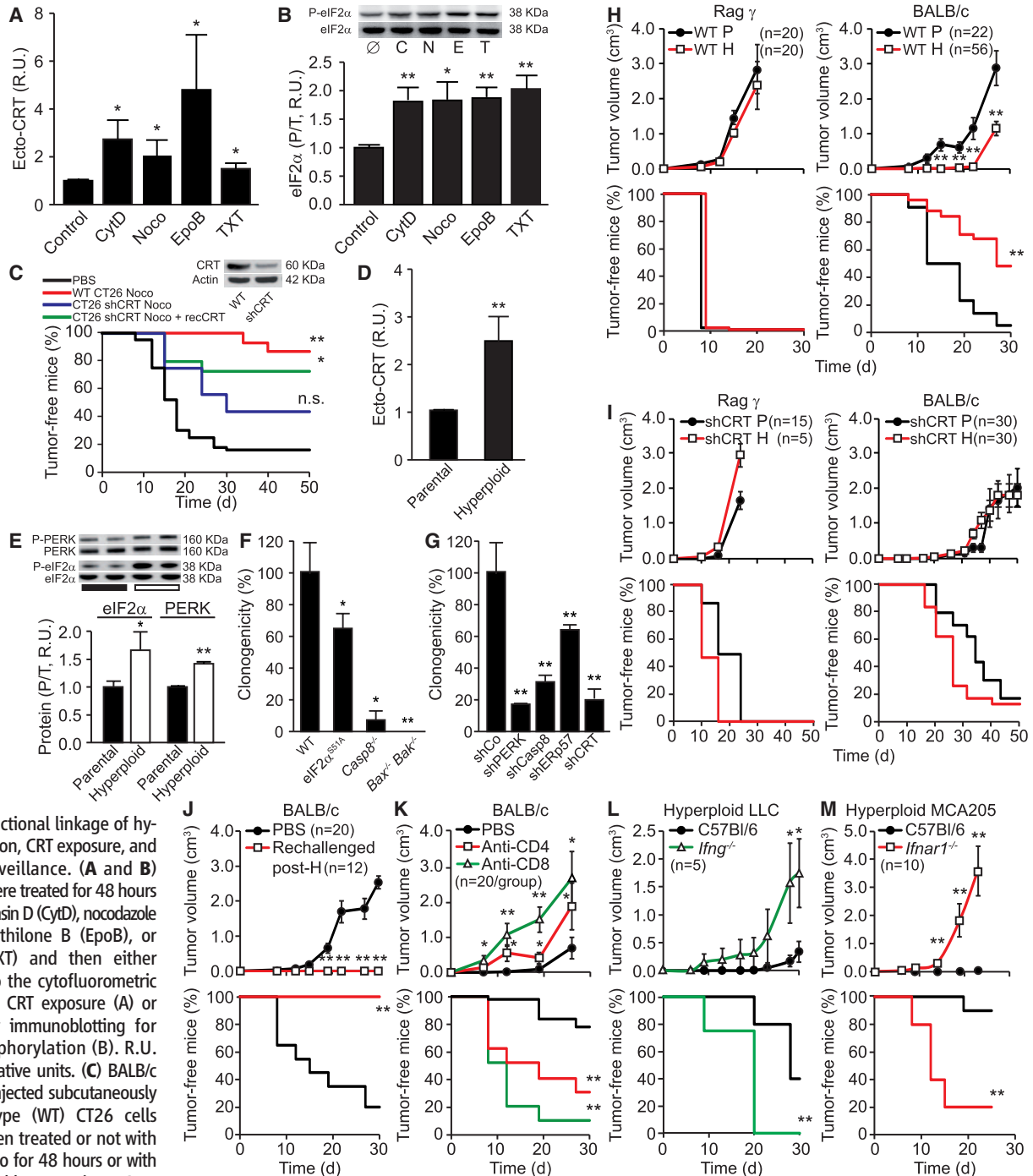


Fig. 1. Functional linkage of hyperploidy, CRT exposure, and immunosurveillance. (A and B) CT26 cells were treated for 48 hours with cytochalasin D (CytD), nocodazole (Noco), epothilone B (EpoB), or taxotere (TXT) and then either subjected to the cytofluorometric detection of CRT exposure (A) or analyzed by immunoblotting for eIF2 α phosphorylation (B). R.U. indicates relative units. (C) BALB/c mice were injected subcutaneously with wild-type (WT) CT26 cells that had been treated or not with 100 nM Noco for 48 hours or with CT26 cells stably expressing a CRT-specific short hairpin RNA (shRNA) that had been preincubated or not with recombinant CRT (recCRT) and treated with 100 nM Noco for 48 hours. In all cases, mice were injected 1 week later with live WT CT26 cells, and tumor incidence was monitored. These experiments were done three times (15 to 20 mice per group). (D) Parental (P) and hyperplod (H) CT26 cells were stained to measure CRT exposure on the cell surface. (E) Constitutive eIF2 α phosphorylation in H CT26 clones (two representative clones of at least $n = 5$), as determined by immunoblotting. Clonogenicity of hyperplod mouse embryonic fibroblasts (MEFs) (F) or CT26 cells (G) upon disruption of the CRT exposure pathway. The cloning efficiency of nocodazole-treated, FACS-purified WT cells or cells transfected with a scrambled control with 8n DNA content was considered as 100%. In vitro data were compared with one-tailed Student's t tests, tumor incidence with the log rank test. * $P < 0.05$; ** $P < 0.01$; n.s., nonsignificant, as compared to untreated P CT26 cells [(A), (B), (D), and (E)], untreated (F), or shCo-transfected H (G) CT26 cells, or mice challenged with phosphate-buffered

saline (PBS) only (C). (H to M) Evidence for an immune response to hyperplod cancer cells. (H) P or H CT26 cell clones were injected into Rag γ or BALB/c mice. (I) Tumor growth of P or H CT26 cells treated with a shRNA (shCRT) to CRT was monitored in Rag γ or BALB/c mice. (J) Growth of P CT26 cells in naïve mice and in tumor-free BALB/c mice previously (2 months before) inoculated with H CT26 cells [as in (H)]. (K) H CT26 cells were inoculated into BALB/c mice that were depleted of CD4 $^{+}$ or CD8 $^{+}$ T lymphocytes by injection of suitable antibodies. (L and M) Contribution of IFN- γ and *Ifnar1* to the immunosurveillance of hyperplod cells. H LLC (L) and MCA205 (M) cells were inoculated into wild-type [(L) and (M)], *Ifng* $^{-/-}$ (L), or *Ifnar1* $^{-/-}$ (M) C57Bl/6 mice. Tumor growth curves [(H) to (M), top graphs] were analyzed with one-tailed Student's t test, whereas tumor incidence [(C) and (H) to (M), bottom graphs, illustrated with Kaplan-Meier curves] was compared by log rank test. Error bars indicate SEM. * $P < 0.05$, ** $P < 0.01$, as compared with P cells (H), PBS-challenged mice [(J) and (K)], or immunocompetent C57Bl/6 mice (M).

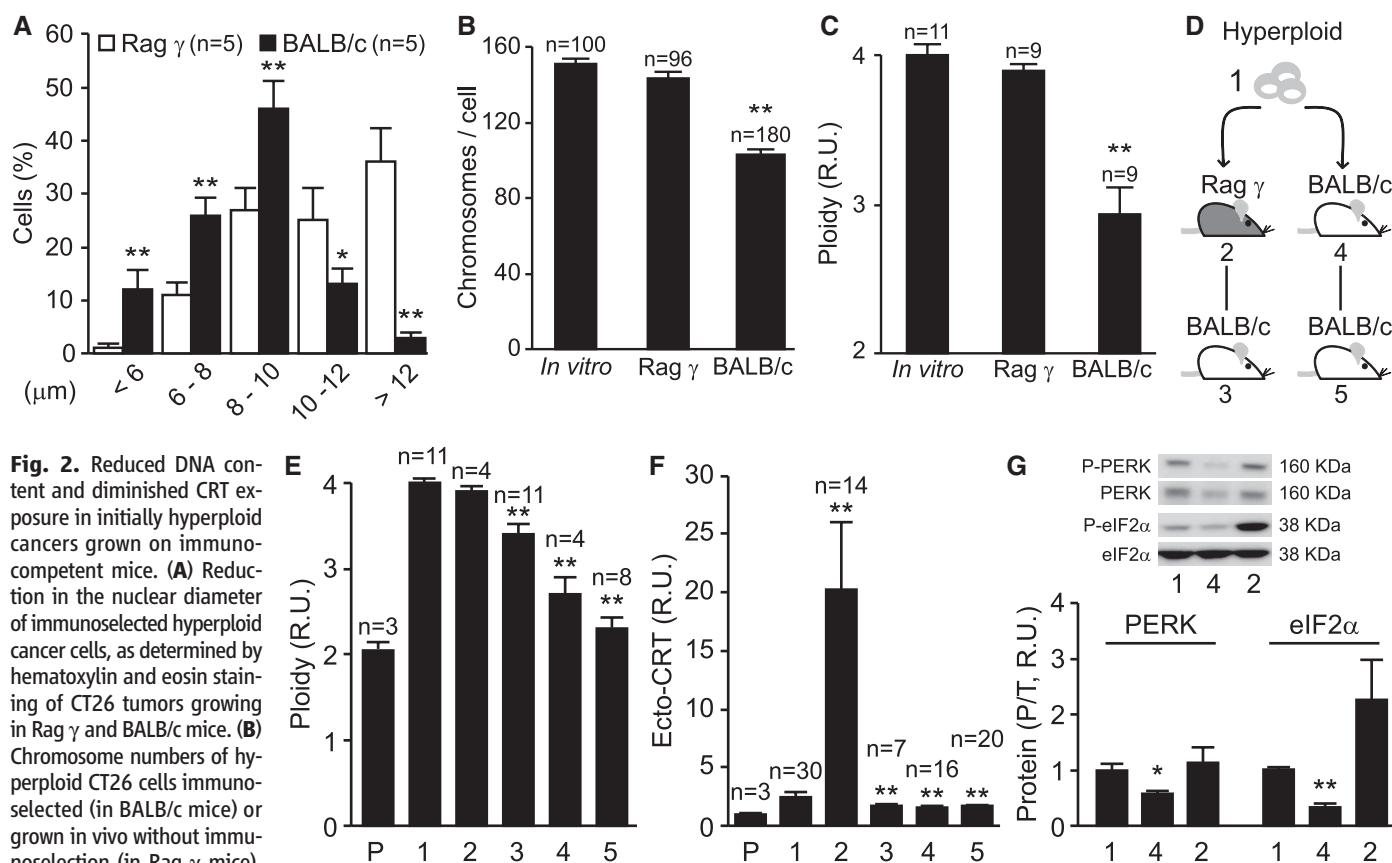
mice (Fig. 2A and fig. S12A). Hence, immunoselection, the passage of cancer cells in immunocompetent (as opposed to immunodeficient) mice, led to a reduction in nuclear size. Accordingly, immunoselected tumors from BALB/c hosts contained fewer chromosomes (Fig. 2B and fig. S12B) than tumors that had grown in vivo without immunoselection (i.e., recovered from Rag γ mice). Immunoselection led to a reduction of overall DNA content (Fig. 2C and fig. S12C) without preferentially affecting a particular set of chromosomes, as determined by comparative genomic hybridization and multicolor fluorescent in situ hybridization (fig. S13 and tables S1 and S2). Similarly, originally hyperploid LLC and MCA205-derived cancers exhibited reduced DNA content upon immunoselection (fig. S14, A and B). The amount of externalized CRT (ecto-CRT) decreased in hyperploid CT26, MCA205, and LLC cancer cells grown in immunocompetent mice yet remained constant or even increased (together with that of ecto-ERp57) upon growth in immunodeficient mice (perhaps because CRT exposure correlates with the fitness of hyperploid cells or because extracellular CRT exerts trophic, pro-

inflammatory, and angiogenic effects) (27). The suppression of CRT exposure correlated with a reduction in ploidy, as well as with decreased eIF2 α phosphorylation. Hyperploid cells recovered from immunodeficient mice (which conserved their hyperploid status and even increased CRT externalization in vivo) exhibited reduced ploidy and lower amounts of ecto-CRT after growth in immunocompetent mice (Fig. 2, D to G, and fig. S14, C to E). These results confirm the strong correlation between CRT exposure and hyperploidy.

We also investigated whether immunoselection may influence ploidy in spontaneous or genetically or chemically induced cancers. Indeed, carcinogen- or *Myc*-driven tumors exhibited larger nuclei and higher levels of eIF2 α phosphorylation in immunodeficient (Rag γ , *Stat1*^{-/-}, or *Dnam1*^{-/-}) (28) mice than in immunocompetent hosts (Fig. 3, A to D, and fig. S15, A to D). To investigate the translational relevance of these findings, we determined the ploidy status and ER stress response on breast cancer tissues from 60 patients (table S3) with locally advanced breast cancer. In this context, the efficacy of neo-adjuvant chemother-

apy is strongly influenced by anticancer immune responses (29–31). Successful chemotherapy reduced the nuclear size of the rare residual carcinoma cells from responders, correlating with an increased ratio of tumor-infiltrating CD8⁺ over immunosuppressive FOXP3⁺ cells. In contrast, tumor cells from nonresponders (who manifested discernible breast cancers in spite of six cycles of chemotherapy) exhibited increased nuclear size and increased phosphorylation of eIF2 α (Fig. 3E and fig. S15E). These results correlate a local immune response with reduced nuclear size (in responders) and the absence of a local immune response with increased nuclear size (in nonresponders).

Our results underscore the relation between immunoselection, hyperploidy, and ER stress, which can induce CRT exposure. To test whether CRT might trigger immunosurveillance, we transduced diploid CT26 cells with a construct encoding CRT fused at the N terminus to the murine immunoglobulin (Ig) κ -chain leader sequence (which directs the protein to the secretory pathway) and at the C terminus to the platelet-derived growth factor receptor (PDGFR) transmembrane domain



recovered and then subjected to the determination of ploidy (E), CRT exposure (F), or the phosphorylation of PERK and eIF2 α , normalized to controls (G). In (G), representative results and quantitative data (obtained by densitometry on multiple tumors) are shown. One-tailed Student's *t* test was used for statistical comparisons. Error bars indicate SEM. **P* < 0.05, ***P* < 0.01, as compared to tumors untreated Rag γ mice (A), parental (P) CT26 cells cultured in vitro [(B), (C), (E), (F)], or hyperploid CT26 cells cultured in vitro (G).

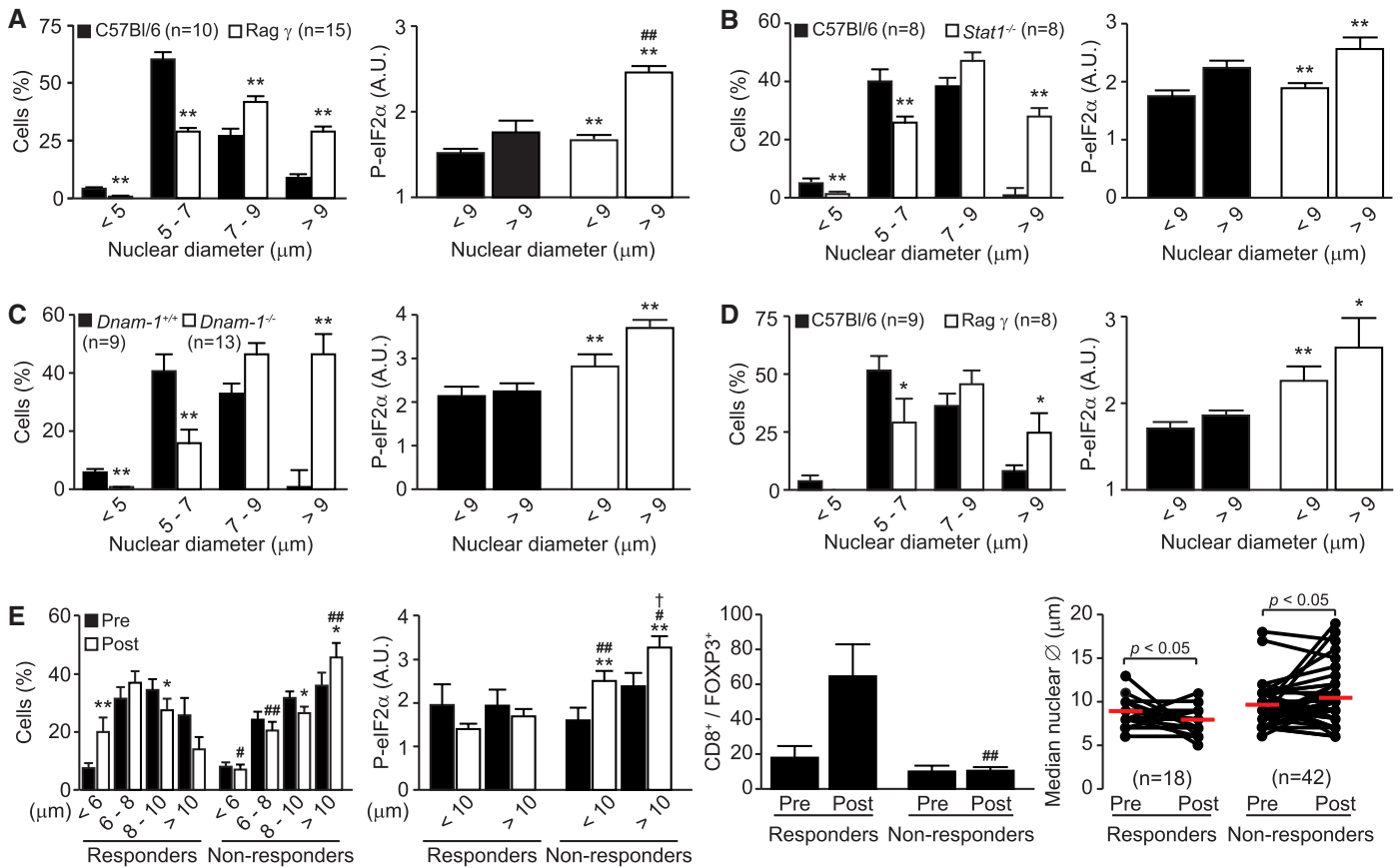


Fig. 3. Immunoselection against hyperploidy and eIF2α phosphorylation. (A to D) Nuclear diameter and eIF2α phosphorylation in methylcholanthrene-induced fibrosarcomas (A and B), *Em-myc*-driven B cell lymphomas (C), or medroxyprogesterone acetate-induced mammary carcinomas (D) from immunodeficient (Rag γ, *Stat1*^{-/-} or *Dnam1*^{-/-}) versus immunocompetent mice. (E) Immunoselection in human mammary adenocarcinomas treated with neoadjuvant chemotherapy. Tumor specimens obtained by surgery from 18 responders and 42 nonresponders before (pre) and after (post) treatment were

stained to determine nuclear diameter, ratio of the number of tumor-infiltrating CD8⁺ to FOXP3⁺ lymphocytes, and eIF2α phosphorylation. One-tailed Student's *t* test was used for statistical comparisons. Error bars indicate SEM. **P* < 0.05, ***P* < 0.01, compared to tumor cells with the same nuclear diameter obtained from immunocompetent mice (A to D) or pretreatment (E). #*P* < 0.05, ##*P* < 0.01, compared to cells from immunodeficient mice with nuclear diameter < 9 μm (A), or to responders (E). †*P* < 0.05, compared to cells with a nuclear diameter < 10 μm from nonresponders (E). A.U., arbitrary units.

(which anchors the protein to the plasma membrane). CT26 cells stably expressing this membrane-exposed CRT [mCRT, which did not affect other surface molecules, including class I MHC (Fig. 4A)] efficiently proliferated in immunodeficient Rag γ mice yet rarely formed tumors in immunocompetent mice (Fig. 4B), and this only occurred coupled to mCRT down-regulation (Fig. 4C), resulting in increased aggressiveness upon reinjection into BALB/c mice (Fig. 4D). Similarly, the reestablishment of high ecto-CRT levels (by mCRT transfection) in immunoselected (former) hyperploid cells limited the aggressiveness of tumor cells that otherwise would have rapidly proliferated upon reinoculation into immunocompetent mice (Fig. 4E). Thus, ER stress-elicited exposure of CRT at the cell surface, as mimicked by mCRT transfection, constitutes a trait of hyperploid cells that can elicit immunosurveillance and is counterselected during tumorigenesis in immunocompetent mice.

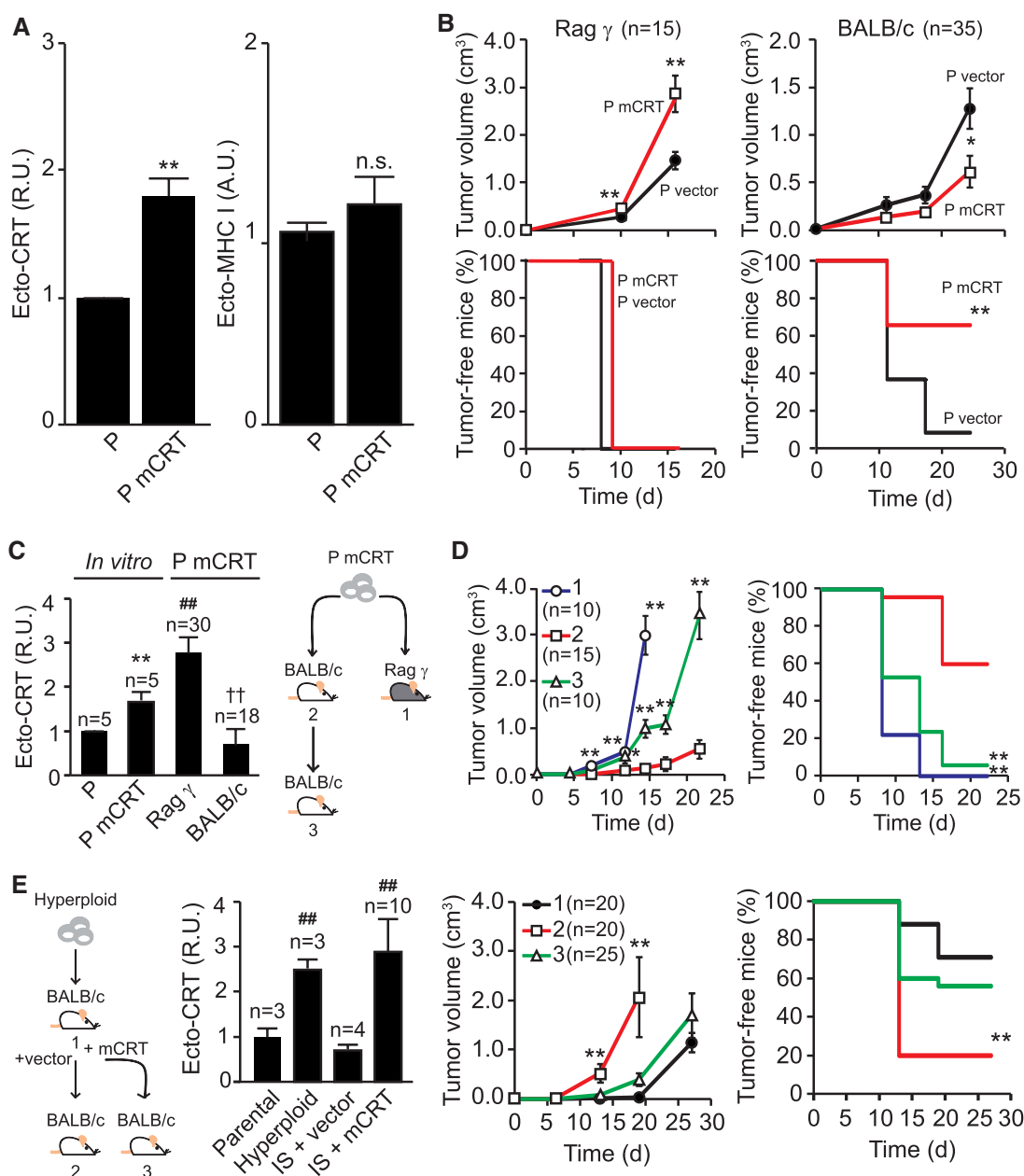
Our results indicate the existence of an immunosurveillance mechanism for the control of

ploidy that involves CD4⁺ and CD8⁺ T lymphocytes, as well as both type I and type II interferons, in line with results from other models of immunosurveillance (32–34). In our model, increases in ploidy were consistently accompanied by ER stress, resulting in the translocation of CRT to the plasma membrane surface. Conversely, immunoselection led to a coordinated reduction in ploidy, ER stress, and CRT exposure, both in established or developing cancers. Transfection-enforced expression of mCRT was sufficient to elicit immunoediting in the absence of hyperploidy. Thus, beyond oncogenic stress that elicits a DNA damage response (and a subsequent increase in the expression of NKG2D ligands and CD95/FAS) (3), hyperploidy-associated ER stress and CRT exposure may contribute to cancer immunosurveillance. Future studies must address which additional factors, such as changes in the expression and presentation of tumor antigens, determine whether a cancer cell is susceptible to or escapes from immunosurveillance.

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Fig. 4. Ecto-CRT underlies the immunogenicity of hyperploid cancer cells. (A to D) Immunosurveillance and immunoselection mechanisms activated by membrane-bound CRT on CT26 cells. Parental (P) CT26 cells were transduced with a construct encoding mCRT or vector only and subjected to immunostaining for the quantification of externalized CRT or MHC class I molecules (A). Alternatively, mCRT-expressing P cells were injected into Rag γ or BALB/c mice, followed by monitoring of tumor growth and incidence (B). Ecto-CRT was measured by immunostaining on recovered cancer cells (C). (D) Tumor growth and incidence of mCRT-expressing P cells sequentially injected into Rag γ (1) or BALB/c (2 and 3) mice. (E) Restoration of immunosurveillance on immunoselected (IS) hyperploid (H) cells by mCRT. H clones were injected into BALB/c mice for immunoselection (1). Then, tumor cells were recovered, transduced with a mCRT-encoding construct (3) or the empty vector (2), analyzed for CRT exposure, and re-injected into BALB/c mice, followed by monitoring of tumor growth and incidence. Experiments were performed three times, yielding comparable results. One-tailed Student's *t* tests were used for statistical comparisons of in vitro data. Tumor growth and incidence (the latter being illustrated with Kaplan-Meier curves) were compared by one-tailed Student's *t* and log rank tests, respectively. Error bars indicate SEM. **P* < 0.05; ***P* < 0.01; n.s., nonsignificant compared with P CT26 cells [(A) to (C)], mCRT-expressing cells (D), or H cells (E) growing for the first time in BALB/c mice. ###*P* < 0.01, compared with mCRT-expressing CT26 cells grown in Rag γ mice (C).



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M.C., and G.K. designed the study. L.S., L.Z., M.C., and G.K. analyzed results. L.S., L.G., and G.K. assembled the figures and wrote the paper. The authors declare no conflicts of interest.

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Mycobacterial Disease and Impaired IFN- γ Immunity in Humans with Inherited ISG15 Deficiency

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may result from single-gene inborn errors of immunity is gaining ground (1–3). From this genetic perspective, one of the most thoroughly investigated pediatric syndromes is Mendelian susceptibility to mycobacterial disease (MSMD), a rare disorder predisposing individuals to severe clinical disease upon infection with weakly virulent mycobacteria, including *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) vaccines (4). These patients are also susceptible to *Salmonella* and *M. tuberculosis* (5, 6). Genetic dissection of MSMD has revealed disease-causing germline mutations in *IFNGR1*, *IFNGR2*, *STAT1*, *IL12B*, *IL12RB1*, *NEMO*, *CYBB*, and *IRF8*, the products of which are involved in interferon- γ (IFN- γ)-mediated immunity (2, 7, 8). There is considerable allelic heterogeneity at these loci, defining 15 distinct genetic disorders. However, the genetic etiology of about half of the cases of MSMD has not been identified.

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The use of the same whole-exome sequencing approach for P2 and his 15-year-old brother (P3), who also has MSMD, led to the identification of 33 previously unreported homozygous variants, including 10 in chromosomal regions linked to MSMD. The best candidate variant was a frameshift insertion in *ISG15* (c.336_337insG/336_337insG). This mutation did not result in a premature stop codon (p.Leu114fs); instead, it potentially leads to the production of a protein that is 187 rather than 165 amino acids in length (Fig. 1, A and B; fig. S1A; and supplementary methods). In both families, the segregation of the *ISG15* mutant alleles was consistent with autosomal recessive MSMD. We also sequenced the *ISG15* coding sequence in 1056 controls from 52 ethnic groups in the HGDP-CEPH human genome diversity cell line panel, as well as 100 Turkish and 100 Iranian additional healthy controls, none of whom carried either of the mutant *ISG15* alleles. Together with their absence in both public and our own databases (table S1), this finding suggests that these two variants are not irrelevant polymorphisms. Finally, none of the known polymorphic variants of *ISG15* are nonsense or frameshift, further suggesting that the two alleles found here may be disease-causing.

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Fig. 1. The familial segregation and expression pattern of the *ISG15* allele indicates recessive inheritance and an absence of protein production. **(A)** Familial segregation in a family from Turkey (Kindred A) and a family from Iran (Kindred B). **(B)** A graphical representation of the proISG15 protein is shown. The LRLRGG ISGylation domain, the eight-amino acid sequence (black) cleaved to yield active ISG15, and the putative proteins synthesized in the patients are shown. **(C)** EBV-B cells from control 1 (C1), control 2 (C2), a *STAT1*^{-/-} patient (negative control), and P1 were left untreated or treated with IFN- α . The cells were then lysed, and the lysates were subjected to Western blotting. **(D)** EBV-B cells from C1, a *STAT1*^{-/-} patient, and P1 were stained with ISG15 and STAT1 antibodies and analyzed by flow cytometry. **(E)** SV-40-fibroblasts from C1, a *STAT1*^{-/-} patient, P1, and P2 were analyzed as in (C). All experiments were performed at least three times.

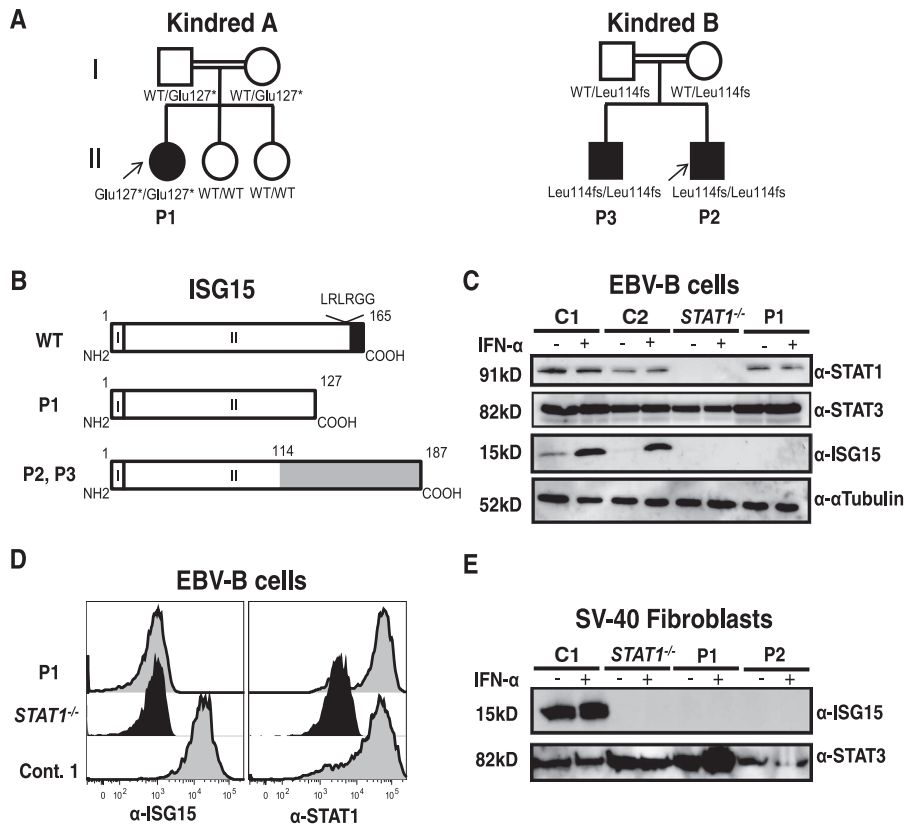
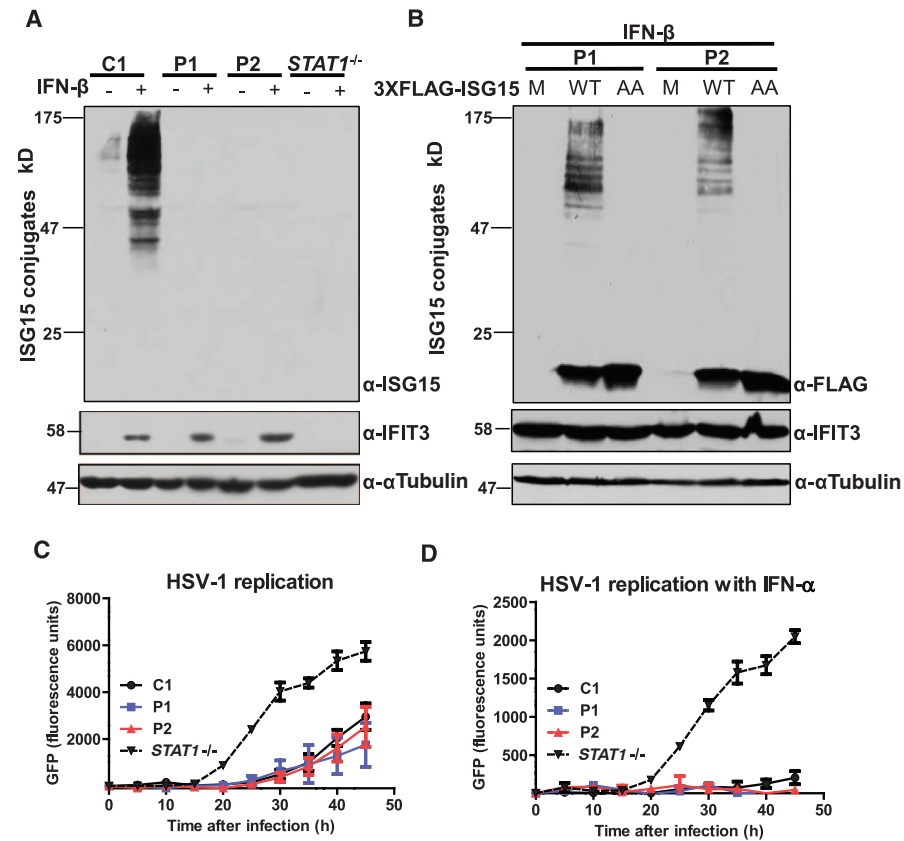


Fig. 2. ISGylation and viral susceptibility in cell lines derived from patients with mutations in *ISG15*. **(A)** Control SV-40-immortalized fibroblasts (C1) and fibroblasts derived from P1, P2, or a *STAT1*^{-/-} patient were either left untreated or treated with IFN- β for 24 hours. Cell extracts were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotting with antibodies against ISG15, IFIT3, or α tubulin. **(B)** SV-40 fibroblast cell lines from patients P1 and P2 were mock-transfected (M), transfected with a plasmid encoding 3XFLAG-ISG15 (WT), or transfected with a plasmid encoding a form of ISG15 unable to conjugate with proteins (AA). Eighteen hours after transfection, we treated the cells with IFN- β for an additional 18 hours. Cell extracts were analyzed by SDS-PAGE and immunoblotting with FLAG, IFIT3, or tubulin antibodies. **(A)** and **(B)** are representative of at least three independent experiments. **(C)** and **(D)** Herpes simplex virus (HSV)-1 replication was monitored by assessing the fluorescence of green fluorescent protein (GFP) fused to a viral capsid protein in SV-40 fibroblasts from a healthy control C1, P1, P2, and a *STAT1*^{-/-} patient, infected with HSV-1 at a multiplicity of infection of 0.2 for the times indicated. Cells were treated with either medium alone (C) or with IFN- α (D) for 24 hours before infection. The results shown are the means of four independent experiments. Error bars indicate SEM.



repeats 3 (IFIT3) (Fig. 2A). These data suggest that the two mutant *ISG15* alleles are loss-of-expression alleles.

We investigated ISG15 production in various leukocytes. Surprisingly, granulocytes had the highest levels of intracellular ISG15, which was detected in control cells but not P1 cells (fig. S2, A and B). Unlike live BCG, IFN- α up-regulated *ISG15* mRNA in all of the leukocyte subsets that we tested (fig. S2C). We then investigated the conjugation of ISG15 to various intracellular proteins (ISGylation) after stimulation with IFN- β . Fibroblasts from P1 and P2 lacked detectable IFN- β -inducible ISGylation (Fig. 2A). The transfection of fibroblasts from P1 and P2 with wild-type (WT) FLAG-ISG15 restored both ISG15 production and ISGylation in this assay, where-

as transfection with the negative control, FLAG-ISG15AA, a mutant that cannot tag proteins, did not restore ISGylation (Fig. 2B). The two human mutant *ISG15* alleles identified in our patients were, therefore, loss-of-expression and loss-of-function (for ISGylation) alleles.

ISG15 is induced by IFN- α/β , which is produced in response to viral infection (13, 14), and ISG15 and ISGylation have been shown to play a role in antiviral defense (12, 15–17). The infectious phenotype of ISG15-deficient mice is characterized by enhanced susceptibility to some, but not all of the viruses tested (18–20). We cannot rule out enhanced susceptibility to other as-yet-unencountered viruses, but the three affected teenagers are at least normally resistant to several common viruses (table S2 and supplementary

materials section 1). We thus assessed the replication and cytopathic effects of three relevant viruses in control cells and cells from the patients (Fig. 2, C and D, and fig. S3, A to H). Both cell viability and viral replication levels were normal, as was the degree of protection afforded by prior treatment with IFN- α . Hence, the lack of a viral phenotype for our patients' cells in vitro is consistent with the lack of severe viral disease in vivo.

Human monocytes and lymphocytes have been shown to secrete the mature form of ISG15 (21). ISG15 secretion has been detected after treatment with IFN- α/β , but the induction of ISG15 secretion by BCG has not been investigated (22). We monitored ISG15 levels within cells and in the supernatants of untreated, BCG-

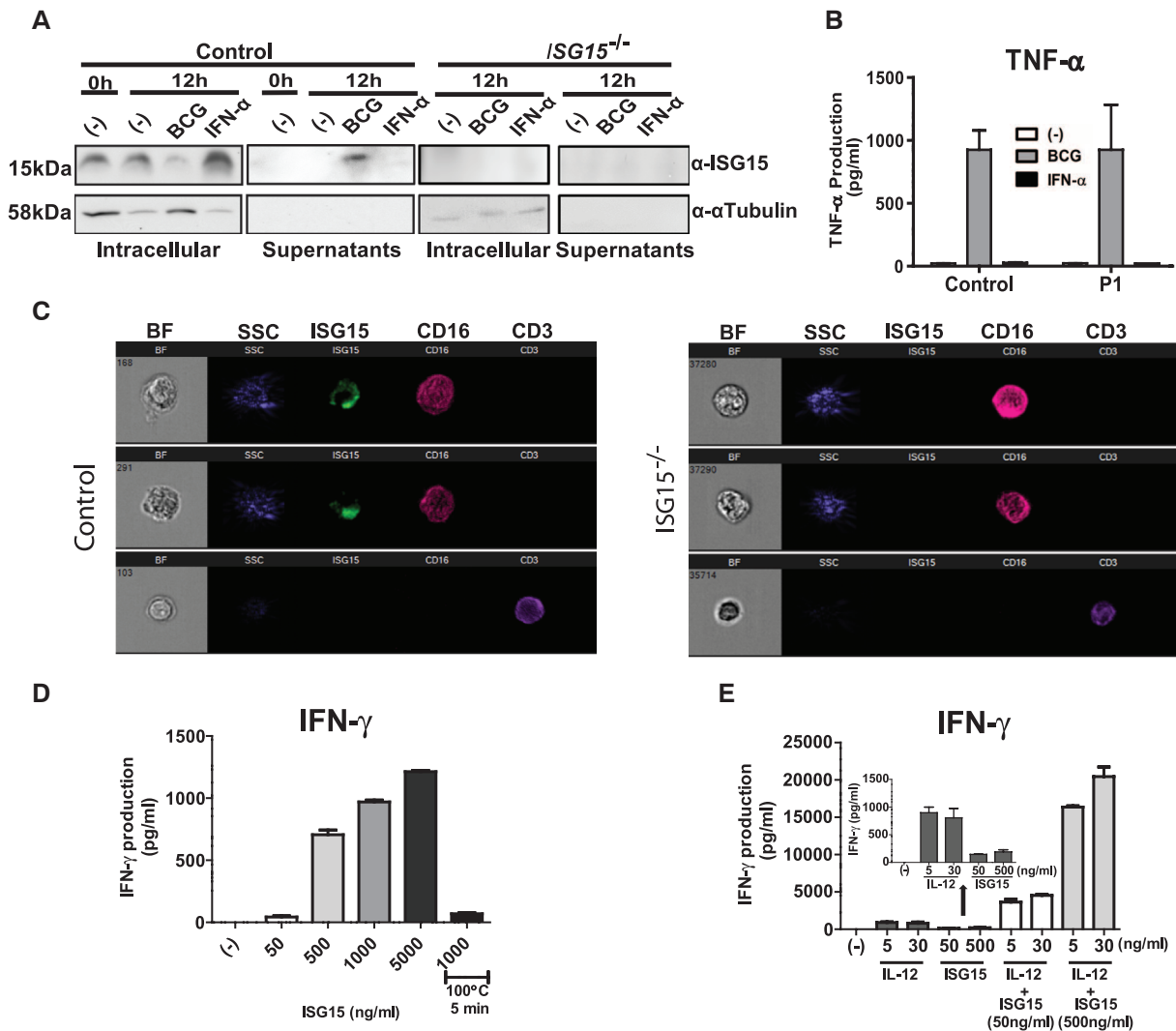


Fig. 3. ISG15 secretion and the induction of IFN- γ production in leukocytes. Control and P1 leukocytes were left unstimulated (-) or were stimulated with BCG and IFN- α 2b. After 0 and 12 hours, cells and supernatants were harvested and subjected to Western blotting (A) or TNF- α enzyme-linked immunosorbent assay (ELISA) (B). (C) Resting control and P1 leukocytes were labeled extracellularly with CD16 and CD3 antibodies and intracellularly with ISG15 antibody and were subjected to ImageStreamX (Amnis, Seattle, WA) analysis

with bright field (BF) and side scatter (SSC) also shown. (D and E) IFN- γ secretion was measured by ELISA in PBMCs stimulated with vehicle (-), various doses of recombinant human ISG15 (including boiled recombinant human ISG15 to exclude LPS contamination) (D), recombinant human ISG15, and IL-12, alone and in combination (E). The IFN- γ secretion results shown in the figure are representative means of at least three independent experiments. Error bars indicate SEM.

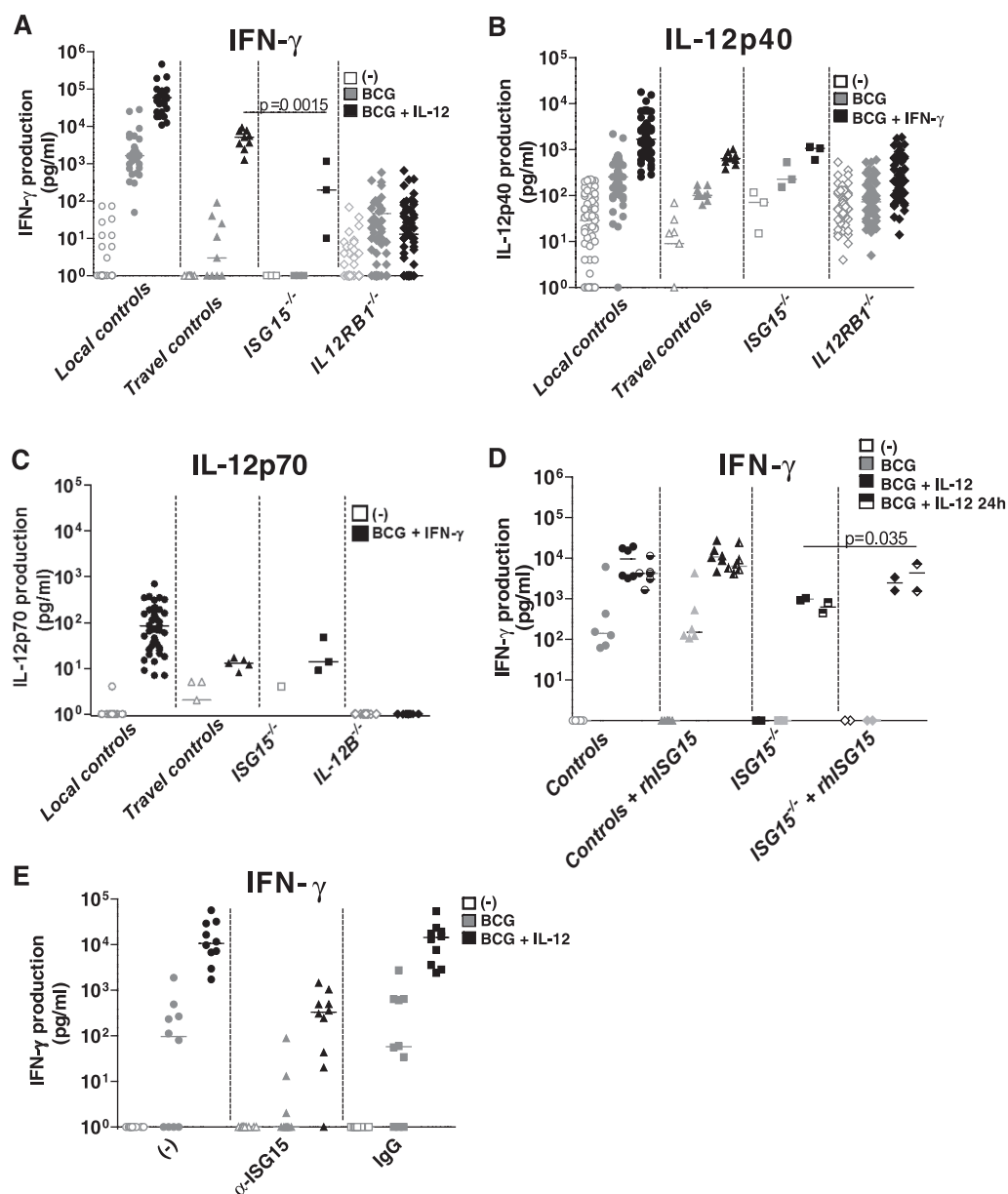
or IFN- α -treated leukocytes (Fig. 3, A to C). BCG induced the release of ISG15 into the supernatants of control leukocytes, with a concomitant decrease in the amount of intracellular ISG15. By contrast, the amount of intracellular ISG15 increased in IFN- α -treated leukocytes. ISG15 was not detectable in P1 leukocytes and supernatants, in any of the conditions tested. Control and P1 leukocytes secreted similar levels of tumor necrosis factor (TNF)- α upon BCG challenge (Fig. 3B). Granulocytes, which displayed the highest levels of intracellular ISG15 expression (fig. S2, A and B), also secreted ISG15 upon BCG or *Staphylococcus aureus* stimulation (fig. S4A). Conversely, bacterial lipopolysaccharide (LPS), like IFN- α , did not trigger ISG15 secretion (fig. S4A).

In granulocytes, ISG15 was localized in gelatinase and secretory but not azurophilic or spe-

cific granules (23) (fig. S4B). Consistent with this finding, ISG15 was not detected in granulocytes from a patient with inherited granule deficiency (fig. S5A). We also detected secreted ISG15 in both IFN- α -treated and untreated control EBV-B cell and SV-40 fibroblast supernatants. By contrast, ISG15 was not detected in the supernatants of cells from P1 and P2 (fig. S5B). The transduction of P1 EBV-B cells with a lentiviral construct encoding WT ISG15 resulted in the restoration of both intracellular and free extracellular ISG15 levels (fig. S5B). Finally, 293T cells transfected with the WT allele but not with the ISG15 allele of P1 produced both intracellular and extracellular proteins (fig. S5C). These experiments suggest that many cell types can secrete ISG15 and that granulocytes, via their secretory pathway, are a major source of extracellular ISG15 during phagocytosis.

Free ISG15 has been shown to induce the secretion of IFN- γ by natural killer (NK) cells and T cells (21, 24, 25). We also observed such an induction of secretion when we stimulated control peripheral blood mononuclear cells (PBMCs) with recombinant human ISG15 (Fig. 3D). We found ISG15 to be effective alone or in synergy with interleukin-12 (IL-12), a cytokine well known to induce IFN- γ (Fig. 3E). Bioactive ISG15 was produced in mammalian cells, and heat-inactivated ISG15 was not bioactive (Fig. 3D). The most responsive cells appeared to be CD3⁺ CD56⁺ NK cells (fig. S5D). We also stimulated PBMCs depleted of NK cells, T cells alone, or NK cells alone. NK cells were the key ISG15-responsive leukocytes, in terms of IFN- γ induction (fig. S5E). The mechanism underlying the synergy between ISG15 and IL-12 was unclear, but ISG15 did not influence the expression of IL-12 receptor chains on

Fig. 4. Impaired IFN- γ production in ISG15^{-/-} patients and rescue by exogenous ISG15. (A to C) Cytokine production in the supernatants of whole-blood cells from local controls ($n = 29$), travel controls ($n = 9$), ISG15^{-/-} ($n = 3$), and IL12RB1^{-/-} patients ($n = 58$), left unstimulated or stimulated with BCG alone or BCG plus cytokine (indicated), as detected by ELISA. (D) Alternatively, recombinant human ISG15 was added at the same time or 24 hours before whole-blood activation by BCG and IL-12. The amounts of cytokine secreted are normalized for 10^6 PBMCs on a logarithmic scale, and medians are indicated by solid bars. Differences in log-transformed IFN- γ levels after stimulation with BCG and IL-12 were assessed (i) between ISG15^{-/-} patients and travel controls in Student's t test (A) and (ii) between ISG15^{-/-} patients before and after adding recombinant human ISG15 by two-way analysis of variance, to account for both activation by recombinant ISG15 and activation time (D). (E) IFN- γ secretion was measured in whole blood stimulated with vehicle (-), BCG, or BCG plus IL-12, in the presence of vehicle, a blocking antibody against ISG15, or an immunoglobulin G (IgG) 1 isotype control.



T and NK cells (fig. S6A). The IFN- γ -inducing activity of ISG15 was not dependent on ISGylation, because IFN- γ was also induced by ISG15 Δ GG (a mutant incapable of conjugation) in combination with IL-12 (fig. S6B).

Whole-blood leukocytes from P1, P2, and P3, like those from IL-12p40- and IL-12R β 1-deficient patients, produced only small amounts of IFN- γ when stimulated with BCG or BCG and IL-12. By contrast, they produced normal amounts of IL-12p40 and IL-12p70 when stimulated with BCG or BCG and IFN- γ (Fig. 4, A to C). The addition of recombinant human ISG15 restored IFN- γ concentrations to almost normal levels (Fig. 4D), a situation reminiscent of the almost complete rescue of IL-12p40 deficiency by IL-12 (26). In P1, both NK and T cells produced small amounts of IFN- γ , as demonstrated by flow cytometry (fig. S7, A and B), and the addition of ISG15 also partially rescued this phenotype (fig. S7B).

We then assessed the contribution of ISG15 to the induction of IFN- γ in whole-blood leukocytes from 10 healthy donors after exposure to BCG or BCG and IL-12 by adding (or not adding) a monoclonal antibody specific for ISG15 (or its isotype control) (Fig. 4E). ISG15 blockade markedly decreased IFN- γ secretion. We monitored the intracellular levels of IFN- γ in T and NK cells in the same conditions. Extracellular ISG15 was required for the induction of IFN- γ in both T and NK cells (fig. S7C). Finally, we showed that *Isg15*^{-/-} mice succumbed earlier than their WT littermates upon infection with *M. tuberculosis* (fig. S8, A and B). Collectively, these experiments suggest that the lack of secreted ISG15 in the three patients with ISG15 deficiency accounts for their low levels of IFN- γ secretion *ex vivo*, and, thus, for their MSMD phenotype *in vivo*.

The apparent differences in antiviral immunity between humans and mice lacking ISG15 may reflect the small overlap of viral infections documented to date. Our findings nevertheless imply that ISGylation and its contribution to antiviral immunity are largely redundant in

humans in the course of natural infections (27). By contrast, as suggested by previous human studies (21, 24, 25), we found that ISG15 was a potent IFN- γ -inducing cytokine playing an essential role in antimycobacterial immunity. Notably, the clinical and immunological phenotypes of ISG15-deficient patients resemble those of patients with IL-12p40 or IL-12R β 1 deficiency, with impaired but not abolished IFN- γ immunity and relatively mild MSMD (28). Like these and other patients with inborn errors of IFN- γ immunity, ISG15-deficient individuals may be prone to *Salmonella* infections and, possibly, to other intracellular infections of macrophages, including tuberculosis (28). The ISG15-IFN- γ circuit, operating principally between granulocytes and NK cells, may therefore be an “innate” complement to the more “adaptive” IL-12-IFN- γ circuit, which operates principally between mononuclear phagocytes and T cells (29).

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1224026/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S8
Tables S1 and S2
References

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Science Careers

From the journal *Science*



Medical University of South Carolina Dean, College of Graduate Studies



The Medical University of South Carolina (MUSC) invites nominations and applications for the position of Dean, College of Graduate Studies. Located in historic downtown Charleston, SC, MUSC is home to approximately 1,200 health professionals and basic biomedical scientists. Research-generated income exceeds over \$230 million annually with research facilities housing state-of-the-art laboratories and interdisciplinary centers. The College of Graduate Studies offers Ph.D. programs in Biomedical Sciences and in Nursing, combined PhD programs with medicine (MSTP), dental medicine (DMSTP), and pharmacy (PharmD/PhD), and Master of Science programs in Clinical Research and Biomedical Sciences. The College also houses the Clemson University-MUSC Bioengineering Alliance, which offers a Ph.D. in Bioengineering. Additional certificate and MS programs are in the planning stages.

The Dean reports to the Vice President for Academic Affairs and Provost and as chief academic and administrative officer of the College of Graduate Studies is responsible for the educational, research and service missions of the College. As a member of the Provost's leadership team, the Dean will be engaged in all aspects of the institution's vision and mission, be a strong advocate for graduate education, work collaboratively with the deans of the five other Colleges (Dental Medicine, Health Professions, Medicine, Nursing and Pharmacy) and promote an academic enterprise that is innovative, entrepreneurial, diverse and global in its reach.

Applicants or nominees will hold an earned Ph.D. degree in one of the biomedical sciences and will have academic credentials commensurate with appointment as a full professor with tenure, a distinguished record of research productivity, teaching excellence, and administrative experience. Successful candidates will have an ongoing federally funded research program in a biomedical sciences discipline, excellent interpersonal communication skills, strong potential to attract financial support for the college and the ability to foster interprofessional collaboration.

Review of applications will begin **October 15th, 2012** and will continue until the position is filled. All applications and nominations will be submitted electronically at <https://www.jobs.musc.edu/applicants/jsp/shared/frameSet/FrameSet.jsp?time=1346871554548> and should include a letter of application, a comprehensive resume, and a list of five references. All correspondence will be strictly confidential. For more information or nominations contact **Dr. Rick Schnellmann, Chair, College of Graduate Studies Search Committee** at (843) 792-3754 or schnell@musc.edu.

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Applicants must demonstrate high proficiency in statistical computing, with SAS experience strongly preferred. The candidate will be expected to participate in a clinical research service program that advises students, residents and faculty on data analysis and experimental design, provides analysis services and writes analysis plans for grant proposals. The successful candidate will also be expected to participate in the education and training of students in the Master of Public Health program, and potentially students in other health related disciplines. The ideal candidate will have a documented history of successful teaching.

Applicants should submit a letter of application summarizing interests and qualifications, a current curriculum vitae, and the names, addresses and telephone numbers of three references. Review of applications and interviews will begin in November, 2012. **Please send cover letter and curriculum vitae to:**

Michelle Hammer

Program Administrator

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Focus on China

Focus on China: A Tale of Two Cities

As the Chinese government invests increasing amounts of money into scientific research, and biotech multinationals rush to open offices in China, opportunities for scientists are exploding. Two cities stand at the forefront of Chinese science. Beijing, close to the center of power, offers more in terms of academia, government jobs, and research. By contrast Shanghai, China's commercial hub, is home to the lion's share of biotech. Chinese scientists who studied abroad are flocking to both cities to contribute to China's scientific effort, a key pillar of its socioeconomic development. International scientists, seeing a chance to further their careers in an expanding market fueled by vast manpower and funding, and eager for a cultural adventure, are beginning to join them. China is a land of diverse opportunities and myriad challenges; from those already there, objectives, ideas, and advice vary. One thing is certain: The world's most populous nation and second-largest economy is fast becoming a global hub for scientists of the future. **By Clarissa Sebag-Montefiore**



Zhu Yi Zhun, dean of the School of Pharmacy at Shanghai's Fudan University, is one of China's most successful returnee scientists. In 2009, his status was cemented by winning the National Award for Innovative Research Work of the Returnees from the Chinese State Council. After a promising career abroad, Zhu had come back to help spur the Chinese economy via science and to further his own career.

"Coming back to China gave huge opportunities, especially for drug discovery," says Zhu, who completed postdoctoral training at the Institute of Pharmacology at Germany's University of Kiel. His work experience includes stints at the University of Washington in Seattle and the Hoechst Marion Roussel Pharma AG (now Sanofi-Aventis) in Singapore. "The Chinese government has started the drug discovery program for the last few years with big funding."

Zhu, who holds several U.S., Patent Cooperation Treaty (PCT), and Chinese patents for new drugs and has seen one cardioprotective drug successfully launched in Singapore, received grants for more than 160 million yuan (over US\$25 million) in China. Such generous funding, combined with the prospect of furthering science in an exciting, emerging market, was hard to turn down.

OPPORTUNITIES AND GROWTH

China is the world's fastest-growing economy. As the country



expands its scientific endeavors to match its economic prowess, many Chinese nationals are returning home—with a significant number heading to Beijing and Shanghai—to take advantage of ballooning opportunities. A career in China is also attracting a number of international scientists who are tempted by a cultural adventure combined with access to ample funding.

The impetus comes from the very top. The Chinese government regards science as an important solution to China's problems and an engine for the country's expansion. Unlike many other countries, China has the muscle, funds, and, increasingly, talent to ensure science remains a priority. Above all, China is aiming to transform itself from a labor-based economy to an innovation-orientated nation by 2020, as outlined in its 2006–2020 Medium and Long-Term Plan for the Development of Science and Technology. *continued*>

UPCOMING FEATURES

Neuroscience Careers—October 5

Top Employers Survey (print edition)—October 19

European Regional Focus—November 9

Faculty Positions at University of Science and Technology of China (USTC)

The University of Science and Technology of China, is one of the most prestigious universities in China and the only university jointly endowed by both the Chinese Academy of Sciences and the Ministry of Education of China. USTC is widely recognized for its tradition on always placing its researchers and students first, and enormous freedom in choosing research subjects and directions. Small as it is (approx. 1100 faculty members), USTC encompasses a great number of internationally visible scientists covering most frontier areas in science and engineering. Numerous USTC alumni have become world-renowned scientists and entrepreneurs.



► Academic Disciplines and Positions:

The university especially encourages research that requires a multi-disciplinary and non-traditional approach. Successful applicants are expected to lead or establish new research directions at USTC, such as next generation automotive engineering and nuclear engineering. Full-time positions include full professor/associate professor, assistant professor, and post-doctoral researcher. Candidates with remarkably strong academic backgrounds are encouraged to apply for professorship of the recruitment programs. Part-time positions are available for world-class scholars, who will use their sabbatical time to undertake research at USTC.

► Recruitment Programs:

1000Plan Professorship for Chinese Experts (Full Time)

Applicants should be ① Chinese scholars under the age of 55

- ② professors or in equivalent positions in world-renowned research institutions

1000Plan Professorship for Non-Chinese Experts (Full Time)

Applicants should be ① non-Chinese scholars under the age of 65

- ② professors or in equivalent positions in world-renowned research institutions

1000Plan Professorship (Part Time)

Applicants should be ① chair professors or in equivalent positions in world-renowned research institutions at present

- ② able to spend no less than two months in USTC each year during the term of appointment

1000Plan Professorship for Young Talents (Full-time)

Applicants should be ① be young scholars under the age of 40

- ② have a doctoral degree obtained in a world-renowned university
- ③ have no less than three years of post-doctoral research experience (Exemptions on work experience may be applied to those who have made distinguished research achievements in their doctoral studies)

► Support, Salary and Benefits:

The USTC is featured with and proud of its highly collegial, interactive, and supportive environment for researchers. Besides, it offers competitive salary, benefits and start-up package among all the "C9 League" universities of China. The benefits include spacious housing, subsidized faculty dining, premium medical services, etc. Professors of the recruitment programs may receive supplementary remuneration, as well as assistance on the establishment of a dedicated research team. The start-up package includes adequate start-up funds, newly renovated office, ample laboratory space, and plenty of research assistants. Please visit the USTC Talent Recruitment and Services website for more details: <http://employment.ustc.edu.cn/en>.

► Contact Us:

Qualified applicants are invited to email the job intention letter and CV to: job@ustc.edu.cn.

Mailing Address:

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中国科学技术大学
University of Science and Technology of China

State Key Laboratory of Reproductive Biology

Founded in 1991, the State Key Laboratory of Reproductive Biology (SKLRB) at the Institute of Zoology, Chinese Academy of Sciences (CAS) has pioneered the research and training of young generation scientists in the field of reproductive biology. Leading scientists work together to create a center of excellence in the companion fields of reproductive biology and stem cell research. The mission of SKLRB is to advance the science in these fields and foster the translation of fundamental discovery into practical applications of the relevant technologies. Scientists from multiple disciplines interact, collaborate and share expertise and resources, supported by efficient and comprehensive institutional administration. The SKLRB also benefits from its close collaboration with the Beijing Institutes of Life Science, located at the beautiful Campus of Beijing Olympic Park.

Research Directions:

Currently, SKLRB is committed to basic science and translational medicine in reproductive and stem cell biology in the following areas: 1) Fate Specification of Germline Stem Cells, 2) Meiosis and Gametogenesis, 3) Fertilization, 4) Embryo Development and Implantation, 5) Placental Development and Diseases, and 6) Stem Cell Research and Regenerative Medicine. We encourage innovative and challenging projects, extensive collaborations, and long-term research initiatives.

Positions:

Full-time PROFESSOR positions are available in, but not limited to, the following directions: 1) Meiosis and Cell Cycle Regulation, 2) Developmental and Reproductive Immunology, and 3) Stem Cell Biology and Regenerative Medicine.

Qualification and Responsibilities:

Applicants must have a doctoral degree, strong academic background with strong publication records, and excellent communication skills. The successful candidate will serve as an independent group leader.

Salaries and Benefits:

A generous starting package will be offered based on the applicant's qualification and experience, including salary and benefits, housing, and competitive start-up research funds. Candidates with remarkably strong academic backgrounds are encouraged to apply for the fellowship supported by "CAS Hundred Talents Program", "National Thousand Young Talents Program" or "National Thousand Talents Program", respectively, through the SKLRB.

Application Materials:

Please submit your application materials in one package with 1) a cover letter, including your research achievements and future research plan, 2) a curriculum vitae, and 3) contact information of three references to Dr. Qi Zhou (1-5 Beichen West Road, Chaoyang District, Beijing 100101, P. R. China. Email: qzhou@ioz.ac.cn; Telephone: +86-10-64807312).

State Key Laboratory of Reproductive Medicine

Established in 2011 in Nanjing, Jiangsu province, China, on the campus of Nanjing Medical University, the State Key Laboratory of Reproductive Medicine (SKLRM) is a Chinese Ministry of Science and Technology designated national center for research on reproductive medicine. The mission of SKLRM is, to integrate outstanding research in both basic and clinical reproductive sciences and to improve reproductive health and life quality of Chinese population. With strong support from both central and local governments, favorable administrative policies from the University, SKLRM is becoming a leading center in reproductive medicine research, with state-of-art research facility, advanced research platforms, and a fast-expanding research team. To further the development towards a world-class center in reproductive medicine, SKLRM seeks application for multiple faculty positions at ranks ranging from associate professor, full professor to a special rank of full professorship sponsored by China "National Thousand Talents Program".

Research Directions:

Research at SKLRM is centered on key problems of human reproductive health. The major research area are: 1) mechanisms underlying defective gametogenesis; 2) effects of environment-gene interaction on gametogenesis; 3) assisted reproductive technologies (ART) and the health of ART offspring; 4) molecular mechanisms of reproduction-related diseases (e.g., reproductive system cancer, birth defects, metabolic disorders, and reproductive abnormalities); 5) reproductive hormone control of the target organs. More information about SKLRM and its associated faculty can be found at: <http://reprod.njmu.edu.cn/keylab/>.

Positions:

Multiple full-time positions at ranks ranging from associate to full professorship, and positions of a special professorship sponsored by China "National Thousand Talents Program" are open.

Qualification and Responsibilities:

Applicants must have a Ph.D, M.D, or equivalent degree, good communication skills, extensive research training and experience in the relevant disciplines, in addition to an excellent publication record. Faculty at SKLRM is expected to develop a vigorous research program, to obtain grant funding, and to train graduate students with a minimum teaching requirement.

Salaries and Benefits:

A generous starting package will be provided which includes research starting fund, and subsidies for housing and relocation. Salary and other benefits are commensurate with qualifications and experience.

Application Materials:

To apply, please submit a complete CV, a Cover Letter and 3 representative publications to Professors Jiahao Sha (shajh@njmu.edu.cn) or Ran Huo (huoran@njmu.edu.cn) by e-mail. Or mail your application package to the following address: Xianzhi Building Room 708, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210009, P.R. China. Applicants are also required to arrange for two letters of recommendation to be sent directly to the above addresses. Person to contact: Professor Ran Huo, Telephone: 86-025-86862038.

生殖医学国家重点实验室



Focus on China

The numbers speak for themselves. In figures released during this year's annual National People's Congress (NPC), China announced in a draft budget that it would put aside 32.45 billion yuan (US\$5.14 billion) for basic research in 2012—a 26% rise from the previous year. Government spending overall on science and technology is due to rise 12.4% to 228.54 billion yuan (US\$36.23 billion). China is now the world's second leading producer of research. Only the United States beats China in the volume of scientific papers published—and predictions show that it could overtake the United States by as early as 2013.

“Chinese science budgets are expanding, not static or declining like in many other major countries,” says **Ben Bravery**, founder of Kexue Communications, a Beijing-based science communications firm that acts as a bridge between China and the West, working with Chinese researchers, organizations, and educational institutes to transfer knowledge. “Universities and the Chinese Academy of Sciences (CAS) are playing a bigger part on the global stage, faculties are growing, and scientific output is growing too. CAS comprises around 80,000 people—that is enormous compared to other top scientific agencies. Scientists that emerged from the Cultural Revolution are beginning to retire and are making way for younger talent to rise through the ranks and also for overseas Chinese to return to researching in China.”

SCIENTIFIC CULTURE

China's two leading cities, Beijing and Shanghai, are both hubs for scientific research. Yet they demonstrate significant differences in scientific culture.

Beijing, China's capital, houses the country's two top universities, Tsinghua University and Peking University (known colloquially as Beida). As the seat of the government, Beijing is the location chosen for many academic conferences, meetings, and grant reviews and is China's political, cultural, and educational center. By contrast Shanghai, located in the Yangtze River delta, is China's commercial and financial center. While Shanghai also contains many top institutions (including the renowned Fudan University), it is home to the lion's share of the country's biotechnology companies.

“Shanghai has traditionally had a greater commercial focus and I'd



“[China is a] hugely exciting place to be—that's part of the draw. You can't come here without a sense of adventure.”

—Babak Javid

say it is natural that this has spread to modes of scientific inquiry and their outputs,” comments Kexue Communications' Bravery. “Beijing is home to the two most well-respected universities and also houses the majority of the CAS. The vast majority of national institutes, academy bodies, and key research centers are located in Beijing. The CAS does an enormous amount of pure and basic research, and so naturally, most of this takes place in Beijing.”

Despite this, Bravery states that the biggest difference between the two hubs comes down to lifestyle. Both cities are vast metropolises but they offer a very different living experience. While the northern capital suffers from pollution issues and traffic jams, it is considered more “Chinese” and is renowned for its music, art, performance arts, and historical heritage. Shanghai, by contrast, is a “softer,” more international experience, with a larger expat community, a myriad of international restaurants, and an emphasis on fashion, finance, and technology. As the mainland's most globalized city, Shanghai is a place where many foreigners feel more at home.

CULTURAL CHALLENGES

Zhu is not the only scientist to have given up high-profile opportunities elsewhere in favor of a career in China. Before arriving in China, British-Iranian infectious diseases specialist **Babak Javid** was offered a faculty position at a top British university. Javid, who did his B.A., Ph.D., and M.B. B.Chir. at Cambridge in the United Kingdom and was a visiting scientist at the Harvard School of Public Health, turned down the offer. Instead, he took up a post as a professor at the Tsinghua University School of Medicine in Beijing (he remains a visiting senior fellow at Cambridge) and became the first non-Chinese member of the faculty. (A second international faculty member, a Spanish biologist, is arriving in September.)

Javid signed up for a six-year tenure-track position and moved to China with his wife and small daughter in 2012. He was attracted by startup grant funding that was by far the most generous he received (well in excess of US\$1 million over five years) and the intellectual and cultural stimulation of working in a different environment.

“For some time as a family we've been thinking about doing something different,” says Javid. “My wife is very mobile *continued*»



Hiring Professors at All Ranks at South University of Science and Technology (SUSTC) Shenzhen, China

The South University of Science and Technology (SUSTC) invites applications and nominations for all ranks of tenured and tenure-track faculty members in the Division of Science, Division of Engineering and Division of Management & Finance.

SUSTC, officially established in April 2012, is a public institution funded by the municipal of Shenzhen, a special economic zone city in southern China. The University is accredited by the Ministry of Education, China and is a pioneer in higher education reform in China. Set on five hundred acres of wooded landscape in the picturesque Nanshan (South Mountain) area, the new campus offers an idyllic environment suitable for learning and scholarship. SUSTC engages in basic and problem-solving research of lasting impact to benefit society and mankind.

The Division of Science, Division of Engineering, and the Division of Management & Finance wish to hire faculty members at all ranks. Key areas include but not limited to: Neural and Cognitive Sciences, Biology and Gene Engineering, Modern Physics, Control and Modification of Materials, Nanoscience and Nanotechnology, Mathematics and Applied Mathematics, Molecular Chemistry and Catalysis, Large-Scale Computational Research, Robotics and Artificial Intelligence, Information Systems and Electronic Engineering, Modern Cities and Future Developments, Energy Sciences and Technology, Environmental Sciences, Financial Mathematics and Management Sciences. The Divisions especially encourage research that requires a multi-disciplinary approach. Experienced researchers whose interests do not fall within the above areas are invited to suggest new areas of research. **Cluster hiring is possible, with senior members accompanied by junior members in a group.**

The teaching language at SUSTC is English or Putonghua. The choice is made by the instructor. As we expect an international faculty, the majority of teaching materials and reference books will be in English and many classes will be conducted in English. With a very high faculty-to-student ratio, SUSTC is committed to delivering a student-centered education and encourages students to develop their innovative spirits. Students at junior and senior years are expected to participate in research in the Research Centers.

The University offers competitive salaries, fringe benefits including medical insurance, retirement and housing subsidy. Leading Professors, Chair Professors and Professors will be appointed with tenure. Associate Professors and Assistant Professors will be offered tenure-track contracts.

Send nominations, inquiries and applications to: hiring@sustc.edu.cn. All applications should include a CV and a detailed list of publications. **Those interested in cluster hiring should send CVs and publication lists as a group.** Evaluations will commence immediately and appointments will be made on a continuous basis. Additional information on SUSTC is available on the University homepage <http://www.sustc.edu.cn> and <http://english.sina.com/china/2012/0902/502496.html>.



Focus on China

(a piano teacher), but I didn't want to compromise my scientific career. I saw an ad for a job here. And on a whim I spoke to my wife about it. She said: 'This is it, this is what we need to be doing.'" China, Javid enthuses, is a "hugely exciting place to be—that's part of the draw. You can't come here without a sense of adventure."

Despite this, the challenges are myriad. In Beijing, where the architecture is mostly traditional Chinese and Stalinist Soviet, a crucial criterion for Javid was to find a home with a Western-style kitchen and bathroom for his family. Language is also a challenge. Javid's lab personnel speak English, but as a non-Chinese speaker he needs help from local intermediaries for day-to-day tasks such as writing grants for government funds and ordering DNA for sequencing. Cultural differences are apparent from small inconveniences (there is no distilled water on tap in Tsinghua's labs) to larger attitudes. Science in China is rigidly hierarchal: positions of authority are lionized and lab members are treated as employees who are expected to do long hours. "Something I really struggle with is showing my students that spending 24 hours in a lab is not efficient or useful," says Javid, who adds that students often need more "hand-holding" than their Western counterparts.

"Don't think China is America with slightly different food—it's not," he warns. "Take the challenges and maximize the opportunities, otherwise you are going to get frustrated."

GROWING PAINS

Despite these two cities demonstrating rapid growth, the science sector in China is racked with growing pains. Bigger does not necessarily translate as better. The explosion of the number of scientific papers published is one example. Pressure to produce papers has led to plagiarism and faking of data in some cases. (In 2009 the UK-based journal *Acta Crystallographica Section E* was forced to retract dozens of papers by Chinese scientists who had used falsified data, many of which were produced in Chinese universities.) Quality is often considered second to quantity.

The system for evaluating scientific research is to blame, says **Chen Xiaoya**, president of the Shanghai Institutes for Biological Sciences (SIBS). "The evaluating system has too much pressure—there is too much emphasis on the number of publications [and in] which journal you publish the paper," he explains.

Chen cites grant applications as another cause for concern, stating that too many resources go to a small number of good scientists. "On the one hand this is good; on the other hand this may also hurt those scientists who receive too much budget. Too much budget for young scientists will lead them to do too many things and not focus. We also need more transparency. Since the government is increasing funding, how [should they] distribute it?" he asks.

Another problem is that in many areas of life science, the research and postdoctoral salaries in America and Europe are higher than those in China. "But changes are also happening," says Chen.



"SIBS is making efforts to raise postdoc salaries and has established postdoc scholarships for partnering with international pharmaceutical corporations to encourage research excellence of postdocs at SIBS. And with China's favorable policies for attracting talent, more and more Chinese scientists have returned." —Chen Xiaoya

"For instance, SIBS is making efforts to raise postdoc salaries and has established postdoc scholarships for partnering with international pharmaceutical corporations to encourage research excellence of postdocs at SIBS. And with China's favorable policies for attracting talent, more and more Chinese scientists have returned."

GROWTH OF BIOTECH

Shanghai and Beijing are both strong contenders in the biotech industry. "My impression is that at this particular juncture, there is more drug development going on within companies in Shanghai than Beijing," says **Rahim Rezaie**, a research fellow at the University of Toronto. But he adds: "The biotech/pharmaceutical industry is highly globalized and these geographic distinctions are becoming less significant over time."

Zhao Ruilin spent 10 years in the United States where he earned a Ph.D. in medical engineering and medical physics from the Harvard-MIT Division of Health Sciences and Technology and an MBA degree from the Wharton Business School. The Chinese-born scientist became head of business development of Life Technologies Greater China in 2012. "I always say the major reason I came back is a great, great opportunity," says Zhao, speaking from Life Technologies' Shanghai office.

The Chinese biotech industry is growing at a rapid pace. Areas such as health care, biofuel, epidemic control, *continued*

CREDIT: PHOTO PROVIDED BY CHEN XIAOYA

CHINA RETURNEES

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FOCUS ON CHINA

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Focus on China

biological agriculture, biological energy, and food safety have become top priorities for leadership as China's middle class balloons. As part of its innovation drive, the government is focusing funds on encouraging applied research and pioneering new therapies. In 2010, the value of Chinese biological output surpassed 1.5 trillion yuan (US\$236.8 billion), according to the Ministry of Science and Technology.

"There has been tremendous growth in R&D infrastructure in China in recent years, much of it financed from government sources," says Rezaie, who is a coauthor of a 2008 study titled "Chinese Health Biotech and the Billion-Patient Market." "Over the past few years, most major multinational companies have increased their investments in the sector substantially and are increasingly engaged in R&D activities. Those have not been prevalent in China until recent years. The sector that I see as most exciting is research and discovery, broadly innovation type of jobs."

Discoveries and development activities in biotech tend to be fairly similar across the globe, says Rezaie. Yet China offers a unique workplace and career opportunities for biotech scientists.

"One difference is that there are certain things you can do in China that you can't in major industrialized countries," he explains. Examples include gene technology, where regulations in China



"If you want to have a real understanding of one of the world's most important markets, arguably the most important in five to 10 years, you should come to China." —Siddhartha Kadia

are not as stringent as in the United States. China has made crucial breakthroughs in the field, including creating the world's first commercialized gene therapy product, Gendicine. The modernization of traditional Chinese medicine is another growing field in biotech that is distinctive to China.

Opportunities are myriad. Life Technologies is an example of one multinational company which is expanding rapidly in China. Life Technologies operates in Beijing, Shanghai, Guangzhou, Hong Kong, and Taiwan and today has nearly 1,000 employees in Greater China. In 2011, Life Technologies Greater China grew its revenue by double digits and hired over 300 new staff. This year, they are planning to hire another 200.

"If you want to have a real understanding of one of the world's most important markets, arguably the most important in five to 10 years, you should come to China," says **Siddhartha Kadia**, president of Life Technologies Greater China. "This is the market in life sciences and health care that is going to grow the fastest, more than any other country in the world. Does that mean things are easy? No."

Kadia emphasizes that employees who come from training or previous careers abroad can find fitting into a Chinese business environment difficult. "My observations have been that if somebody comes here expecting a very similar approach to work, that could be quite challenging," he says. "You have to be a problem solver, but in a very different way, to work here. In many ways, government regulations are actually still in the state of definition, so it's a great opportunity for some to shape the landscape, but for others it could be very confusing. You need to be more open minded."

Zhao agrees. One of the largest challenges for the scientist was negotiating the business landscape in China. "I think there are always some differences in terms of culture and how to get business done—the same thing can be said in the same way and carry different meanings. Even if I was born in this country!" he states. Despite this, he says, it is worth it. "It's an explosive opportunity that I wouldn't be able to get in the United States."

Clarissa Sebag-Montefiore is a freelance writer based in Beijing, China.

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Featured Participants

Kexue Communications

www.kexuecommunications.com

Life Technologies Greater China

www.lifetech.com

Shanghai Institutes for Biological Sciences (SIBS)

english.sibs.cas.cn

Tsinghua University School of Medicine

www.tsinghua.edu.cn

University of Toronto

www.utoronto.ca



Recruitment of Global Talents Nanjing University

A key comprehensive university dating from 1902 in China under the direct supervision of the Ministry of Education, NJU was among the first group of universities in the "Project 211" and "Project 985". NJU boasts a highly qualified and experienced team of faculty. Among the more than two thousand faculty members of NJU, there are 32 Academicians of the Chinese Academy of Sciences and Engineering, 89 professors of "Chang Jiang Scholars Program of the Ministry of Education", 85 National Outstanding Youth Fund winners, and 10 national distinguished teachers.

NJU has a broad and balanced range of academic disciplines covering natural science, humanity, engineering, and medicine. It has won 1 first-class award and 16 second-class awards in the National Natural Science Award since 2000, ranking top in China. According to statistics in 2009, NJU's Physics, Astronomy, and Atmospheric Science ranked No.1 nationwide; 17 disciplines including Philosophy, Economics, Sociology, and Foreign Languages and Literature ranked No.5 nationwide; Chemistry, Physics, Material Science, Earth science, Biology and Biochemistry, Clinical Medicine, Engineering, Environmental Science and Ecology, Pharmacology and Toxicology, Animal and Botany, and Mathematics ranked top 1% worldwide.

Recruitment Programs

NJU nowadays invites outstanding scholars of all nationalities for "Thousand Young Talents Program (Full-time/ Part-time)", "Thousand Non-Chinese Talents Program", "Thousand Young Talents Program", "Chang Jiang Scholars Program of the Ministry of Education (Distinguished Professors/ Chair Professors)", and "Deng Feng Scholars Program".

Thousand Talents Program (Full time/ Part time)

Full-time Positions:

This program aims at recruiting world-class scholars (under 55 years old if in a field of natural sciences, and under 60 in a field of humanities and social sciences) as full-time Professors at NJU.

Applicants should have worked either as professors or in equivalent positions in world-renowned universities or research institutes. Successful applicants should spend at least 9 months each year undertaking research at NJU during the term of appointment.

Part-time Positions:

This program aims at recruiting world-class scholars (under 55 years old if in a field of natural sciences, and under 60 in a field of humanities and social sciences) as part-time Professors in NJU. Applicants should work either as professors or in equivalent positions in world-renowned universities or research institutes. Successful applicants should spend at least 3 months each year undertaking research at NJU during the term of appointment.

Thousand Non-Chinese Talents Program

This program is designed for world-class scholars of non-Chinese ethnicity under age of 65. Applicants should work either as professors or in equivalent positions in world-renowned universities or research institutes.

Thousand Young Talents Program

This is one type of "Thousand Talents Program" especially for young scholars under the age of 40. Three plus years of overseas post-doctoral research experience is required if the doctoral degree was awarded overseas, while five plus years required if degree obtained in Mainland China. Special offers are granted to those who have made distinguished research

Chang Jiang Scholars Program of Ministry of Education of China (Distinguished and Professors/ Chair Professors)

Distinguished Professors:

The applicant should be under 45 years old if in a field of natural sciences, and under 55 in a field of humanities and social sciences. Applicants should have worked either as associate professors or in equivalent positions in world-renowned universities or research institutes.

Chair Professors:

Applicants should have worked either as professors or in equivalent positions in world-renowned universities or research institutes. Successful applicants should spend at least 2 months each year undertaking research at NJU during the term of appointment.

Deng Feng Scholars Program (Type A/ Type B)

NJU invites applicants for "Deng Feng Scholars Program" positions in the field of Sciences, technology, medicine, social sciences, art, and humanity. We particularly welcome creative and energetic individuals with strong backgrounds in all of those fields.

Type A:

Applicants in world-renowned universities or research institutes overseas should have worked either as associate professors or in equivalent positions. Applicants in world-renowned universities research institutes in Mainland China should have worked either as professors or in equivalent positions.

Type B:

Applicants should be Chinese scholars under the age of 40. Applicants in world-renowned universities or research institutes overseas should have research experience after obtaining a doctoral or post-doctoral degree. Applicants in world-renowned universities research institutes in Mainland China should have worked either as professors or in equivalent positions.

Salary, Benefits and Start-up Package

NJU offers outstanding scientific resources, abundant start-up funding, competitive salary and living conditions. The benefits include generous housing allowance, medical services at state expense, etc. The start-up package includes adequate start-up funds, newly renovated office, ample laboratory space, and plenty of research assistants. Professors of the specially listed programs may receive supplementary remuneration, as well as assistance on the establishment of a dedicated research team. The positions also offer the opportunity for young scholars to undertake research and studies at the international famous university.

Contact Us

The application period is not limited.

Qualified applicants are invited to apply on line at: <http://rczp.nju.edu.cn/> or send a job intention letter and a curriculum vitae to: rcb@nju.edu.cn

Links:

Nanjing University: <http://www.nju.edu.cn/>

Address: 22 Hankou Road, Nanjing, Jiangsu, China 210093

Telephone: +86-25-83686783/ 89686783

NJU Talents Affairs Office: <http://pd.nju.edu.cn/>

Persons to Contact: LIU Hao, ZHOU Wei, JI Yuhan, YU Chunhong

Fax: +86-25-83307923



Wenzhou University Seeks for Academic Leaders and Discipline Backbones

Wenzhou University (WZU <http://www.wzu.edu.cn>) locates in the coastal city of Wenzhou, Zhejiang Province, Mainland China. Besides 50 undergraduate specialties, WZU has 6 master's degree authorization level disciplines and 36 master's degree authorization disciplines.

WZU now seeks for outstanding scholars and technicians in the following and relevant fields:

- * Physics, Operational Research and Cybernetics, Applied Mathematics, Numerical Mathematics;
- * Organic Chemistry, Inorganic Chemistry, Material Science;
- * Electrical Engineering, Electronics Science and Technology, Communication Engineering, Computer Science and Technology;
- * Ecology, Biological Chemistry and Molecular Biology, Environmental Engineering;
- * Laser Material Processing Technology and Equipment, Laser Detection Technology and System, Optoelectronic Application Technology and System;
- * Materials for Civil Engineering, Construction Engineering, Built Environment, Road and Bridge Engineering, Geotechnical Engineering;
- * Finance, Financial Engineering.

Qualifications

Applicants should hold a Doctoral degree in above-mentioned fields with evidence of prominent researches and leading achievements.

Or, the candidates should demonstrate their experience of leadership, such as an associate professor (or equivalent/higher) position in well-known universities or institutes, or technical professionals of operation and management in international enterprises or institutions.

Remuneration and Benefits

Candidates meeting the requirements of (or those equivalent to) China's State "Thousand Talents Project" or Zhejiang's Provincial "Thousand Talents Project" will be paid about RMB 1 million or RMB 0.5 million per year.

Outstanding doctors: about RMB 100 thousand per year.

WZU respects the individuals and encourages diverse developments, strives to provide even better research platforms and create free and fair academic environments for all the researchers. All successful applicants will be provided with accommodation (or subsidy for accommodation), settling-in allowance, scientific start-up funds, and corresponding facilities.

All the above are negotiable.

Interested persons are welcomed to send application materials to rcb@wzu.edu.cn. For enquiries, please feel free to send emails or call 86-577-86595080.



OPEN FACULTY POSITIONS INSTITUTE OF MOLECULAR BIOLOGY ACADEMIA SINICA, TAIWAN, ROC

One tenure-track faculty position is open for a highly qualified individual to establish independent research programs **in the areas of cell imaging and systems biology**. Outstanding candidates in other areas of molecular and cellular biology will also be considered. Applicants should hold a Ph.D. degree or its equivalent, and postdoctoral research experience is preferred. The successful recruit will be appointed at the levels of **Assistant, Associate, or Full Research Fellows** (equivalent to academic ranks of Assistant, Associate and Full Professors at universities), and receive a generous multi-year start-up package, followed by annual intramural support.

The Institute of Molecular Biology at Academia Sinica (<http://www.imb.sinica.edu.tw/en>) provides an active and stimulating research environment, is well supported by both extramural and long-term intramural funding, and features several core facilities (imaging, microarray, Next Generation Sequencing, RNAi, electrophysiology, FACS, bioinformatics and mouse facilities) that provide state-of-the-art resources and key technical expertise to the Institute's research community. Recent research works were published in top journals such as Science, Nature, and Cell. Currently three Ph.D. programs, with one recruiting international students, are formally affiliated with the Institute. English is the official language for regular seminars and most of the lectures at the Institute, and proficiency in Chinese language is not a prerequisite for application.

Applicants should send their Curriculum Vitae, a description of past research accomplishments and future research plans, and arrange for three letters of recommendation to be sent directly to:

Dr. Meng-Chao Yao, Director
c/o Ms. Vivi Chiang
Institute of Molecular Biology,
Academia Sinica
Taipei, Taiwan 11529, ROC

The selection process will start on **December 31, 2012** until the position is filled. Further information can be obtained from **Ms. Vivi Chiang** at vivi@imb.sinica.edu.tw



中国科学院植物研究所
INSTITUTE OF BOTANY, THE CHINESE ACADEMY OF SCIENCES

Faculty Positions in Institute of Botany, Chinese Academy of Sciences

The Institute of Botany, Chinese Academy of Sciences, is a national research institution dedicated to a wide spectrum of researches in plant ecology and evolution, molecular and cellular biology, and sustainable utilization of plant resources. The Institute invites outstanding scientists to apply for Associate and Full Professor positions in the following areas:

- Plant taxonomy
- Biodiversity
- Grassland and pasture management
- Cell development and physiology
- Photosynthesis
- Plant resources
- Plant signal transduction and epigenetics
- Other fields of integrative botany

Successful candidates are expected to develop vigorous research programs and lead an independent research team. The institute will provide competitive start-up packages, annual operating budget, attractive salary, and housing benefits. A curriculum vitae, a summary of research accomplishments, a list of all publications and five selected full papers, a brief description of future plans, and three letters of reference should be sent to: **Ms. Wenjuan Zhang** (email: zhangwenjuan@ibcas.ac.cn, tel: 86-10-62836602). Screening of applications will begin on **October 1st 2012** and continue until the positions are filled.



江蘇大學
JIANGSU UNIVERSITY

Faculty Positions Jiangsu University (Zhenjiang, Jiangsu, China)

Jiangsu University (UJS) invites applications and nominations for tenured and tenure-track faculty members in the following fields including: **Mechanical Engineering, Power Engineering and Engineering Thermophysics, Agricultural Engineering, Material Science and Engineering, Food Science and Engineering, Environmental Science and Engineering, Electrical Engineering, Electronic Science and Engineering, Information and Communication Engineering, Computer Science and Technology, Control Science and Engineering, Vehicle Engineering, Traffic and Transportation Engineering, Management Science and Engineering, Civil Engineering, Mechanics, Basic Medical Science, Clinical Laboratory Diagnostics, Clinic, Applied Economics, Mathematics, Biology, Optical Engineering, Instrument Science and Technology, Metallurgical Engineering, Chemical Engineering and Technology and Pharmacology.**

UJS: With a history of 110 years, Jiangsu University is a comprehensive university especially with its strength in engineering. Its research fields cover at least 10 primary academic categories that include engineering, nature science, medicine, management, economics, law, literature, philosophy, pedagogy, and art. Currently, it also holds 8 post-doctoral research stations, 9 major academic categories with a variety of disciplines for doctoral degrees that offer 42 Ph.D. programs. In addition, it also holds two national key academic disciplines and one additional discipline as a candidate under cultivation, as well as 3 province leading disciplines. We intend to recruit outstanding talents to join UJS.

All positions are available for **Distinguished Professor, Leading Professor, Full Professor, Associate Professor, and Assistant Professor**, which can also be the candidate for the talent programs such as Global Experts program, Changjiang Scholar Program, Distinguished Professor of Jiangsu Province, High-level Innovation Talents and Innovation Team Leader of Jiangsu Province. A successful candidate should hold a Ph.D. degree and an international recognized research record.

The University offers competitive salaries, fringe benefits including medical/dental insurance, retirement and housing subsidy. The positions are available now. Reviewing of application materials will begin on the **15th October** and will be continue until positions are full.

To know more about the related disciplines and positions, please visit:

<http://www.ujs.edu.cn/pub/xiaonei/rczp>.

Contact: Phone: Mr. Shao +86-511-88789659 or Mr. Feng +86-511-88789658. E-mail: hr@ujs.edu.cn



蘇州大學
SOOCHOW UNIVERSITY

Soochow University Center for Circadian Clocks

Multiple faculty positions in the broad areas of chronobiology at Assistant/ Associate/Full Professor level are available in Soochow University Center for Circadian Clocks (SUCCC) in Suzhou, China.

We are seeking outstanding scholars who use fruit fly, zebrafish, mouse or human to investigate molecular genetic mechanisms underlying circadian regulation, or study regulatory roles of circadian clocks in development, reproduction, metabolism, sleep, behaviors, immune responses or tumorigenesis. We are particularly interested in individuals who study systems biology or epigenetics (including miRNAs) of circadian clocks or employing high-throughput approaches to screen small molecules that affect circadian clocks.

Successful candidates should have a Ph.D. with at least 3-year postdoctoral research experience. Applicants should have high-quality publications, and have the ability to develop and maintain an externally funded research program. Competitive relocation and salary packages, generous start-up funds, and spacious lab space will be provided. Suzhou is one of the most livable cities with deep culture heritage and glaringly modern flavor in the world.

Interested individuals should send a cover letter, an updated CV, a statement of research accomplishments/interests, future research plans, and a list of at least three references to **Han Wang** at wanghan@suda.edu.cn, Soochow University Center for Circadian Clocks, Soochow University, Lake Dushu campus, 199 Ren-Ai Road, Suzhou Industrial Park, Suzhou, Jiangsu 215123, China.



Jiangsu Academy of Agricultural Sciences

Open position as Deputy Director and Full Professor in the Institute of Agricultural Facility and Equipment

Jiangsu Academy of Agricultural Sciences (JAAS) is a professional agricultural research and extension institution that has been established since 1932. JAAS ranks at the top of provincial agricultural academies in China in terms of the comprehensive strength in agriculture. JAAS's headquarter and main research facilities are located in Nanjing, Jiangsu, China.

Currently, JAAS is seeking a deputy director with the rank of full professor in the area of agricultural facility and equipment researches. Applicant should have a commitment to scientific excellence and the enthusiasm, energy, and innovative thinking necessary to lead a dynamic institute with a broad and diverse portfolio. Applicant must possess a faculty position already beyond the assistant professor level in a university or the equivalent position in a research institution. In addition, the candidate should demonstrate excellent records of research accomplishment and has a command of bilingual language for English and Chinese, both in spoken and written.

Successful applicant will be offered a competitive package, including sufficient laboratory space, startup funding, relocation fee and competitive salary commensurate with experience, in addition to a housing allowance, and other employee benefits. Applicant can go to www.jaas.ac.cn for application details.

In addition, more information for other regular faculty positions from JAAS relevant to a variety of disciplines in agriculture is also available at www.jaas.ac.cn.

Contact information

E-mail: rsc-gbk@jaas.ac.cn; Tel: 086-25-84390037



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FACULTY POSITION

Cell Biology Program

The Cell Biology Program, Sloan-Kettering Institute (www.ski.edu) has initiated a search for tenure-track faculty members. We are interested in outstanding individuals who have the potential to develop an innovative, independent research program that complements and enhances our existing strengths.

Candidates with research interests in exciting areas of eukaryotic cell biology, including aspects of stem cell biology, and using a variety of experimental approaches and systems are encouraged to apply.

New faculty will be eligible to hold graduate school appointments in the Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, the Weill Cornell Graduate School of Medical Sciences of Cornell University, as well as the Tri-Institutional MD/PhD Training Program. Sloan-Kettering has an outstanding infrastructure as well as state-of-the-art core facilities resources, and we are expanding our research programs.

The deadline for applications is **November 1, 2012**. Interested candidates should visit <http://facultysearch.ski.edu> to access the on-line faculty application. Please visit the site as soon as possible, as it contains important information regarding the required application materials, including deadlines for submission of letters of reference. Informal inquiries may be sent to **Tiffany Lennon** at lennont@mskcc.org or **Dr. Alan Hall, Chair, Cell Biology Program** at halla@mskcc.org.

facultysearch.ski.edu

MSKCC is an equal opportunity and affirmative action employer committed to diversity and inclusion in all aspects of recruiting and employment. All qualified individuals are encouraged to apply.



CHILDREN'S MEDICAL CENTER
RESEARCH INSTITUTE
AT UT SOUTHWESTERN

Faculty Position in Metabolism Research

The Children's Research Institute (CRI) at the University of Texas-Southwestern Medical Center in Dallas, TX seeks applications for a **tenure-track faculty position in the area of metabolism and disease**. Outstanding investigators at any rank will be considered. Candidates must have a Ph.D., M.D. or equivalent degrees and the ability to direct independently-funded research programs. Areas of specific interest include analysis of metabolism at the cellular level, including metabolomics, metabolic flux analysis, metabolic imaging and mitochondrial biology. In addition to analytical equipment dedicated to the investigator's studies, CRI members will also have access to a metabolomics core with triple-quadrupole mass spectrometry and gas chromatography/mass spectrometry. NMR spectroscopy, ^{13}C dynamic nuclear polarization, a human 7-Tesla MRI and a state-of-the-art mouse metabolic phenotyping facility are also available on campus to provide an unparalleled breadth of metabolic analysis.

The UT-Southwestern Medical Center has a long and distinguished history of excellence in disease-related basic science research. The CRI is a new institute recruiting outstanding individuals dedicated to solving fundamental problems in biology and disease. The CRI is a dynamic, stimulating, and highly collaborative scientific environment. Major areas of focus within the CRI will include stem cell biology, cancer biology, and metabolism.

Please submit a CV, a 2-page summary of past accomplishments and research plans, and ask three references to submit letters by **November 1, 2012** to CRIApplicants@utsouthwestern.edu.

UT Southwestern is an Equal Opportunity/Affirmative Action Employer.



Faculty Positions

The Center for Immunology and Microbial Disease at Albany Medical College invites applications for multiple tenure-track Assistant Professor, Associate Professor, and full Professor positions from individuals who have a doctoral degree, postdoctoral experience, and demonstrated research productivity. Applicants for a senior faculty position should have an internationally-recognized research program in microbiology and/or host-pathogen interactions. The basic science departments at Albany Medical College are organized as interdisciplinary research centers and the Center for Immunology and Microbial Disease has a focus on microbial pathogenesis and immune defense, particularly as related to biothreat agents and emerging infections. Our faculty comprises a highly collaborative research group that this year received \$12.2M in NIH funding, ranking it within the top half of all Microbiology and Immunology programs in the country. The successful candidates will receive attractive start-up packages and will have an opportunity to lead a focus group in new laboratory space, with access to all departmental core facilities including the Center's fully-staffed ABSL-3/BSL-3. We have established a close relationship with the New York State Department of Health Wadsworth Laboratories, providing a diverse environment that is rich in infectious disease expertise. Albany Medical College is located in a mid-sized city within the upstate New York Capital Region, and has easy access to Boston, New York City, and the Adirondack Mountains.

Applicants should send their curriculum vitae, a statement of research plans, and contact information for three references to:

Faculty Search Committee
Center for Immunology and Microbial Disease
Albany Medical College
47 New Scotland Avenue, MC-151
Albany, NY 12208

For further information about the Center, visit:
www.amc.edu/Research/imd

*An Equal Opportunity/Affirmative Action Employer.
Women and minorities are encouraged to apply.*

KOREA UNIVERSITY

Faculty Openings

Korea University, in Seoul, South Korea, seeks candidates to join the faculty in the spring semester of 2013 beginning March 1. We are recruiting enthusiastic, well-trained scholars who wish to refine their teaching experience and research in this part of the world. Korea University, founded in 1905, is ranked one of the top universities in Korea by major global assessment reports. The positions available for the candidates are assistant, associate, or full professors (a two or three year tenure track appointment, OR non-tenure track appointment). All candidates are expected to have ample university-level teaching experience, a strong commitment to excellence in scholarship, and dedication to teaching and research in their fields.

[Online Application] - Applicants are asked to submit their applications electronically by using the website at <http://kuweb.korea.ac.kr/faculty>
- Application Deadline: October 24, 2012

[Point of Contact] - Phone : +82-2-3290-1072
- Fax : +82-2-929-9164
- E-mail : faculty@korea.ac.kr



KOREA
UNIVERSITY

We're improving agricultural sustainability through our innovations*



The Syngenta logo is displayed in blue text with a green leaf icon above the 'y'.



A photograph of three female scientists in white lab coats and safety glasses working in a greenhouse. They are looking at a clipboard. In the foreground, there are rows of potted plants on a metal frame.

***Our work matters**

R&D is at the heart of Syngenta which is in a strong growth phase. We are looking to recruit scientists from a broad variety of scientific disciplines including agronomy, biology, chemistry, biochemistry, breeding and genetics. Syngenta is also proud to be ranked among the Top Employers according to Science's 2012 Top Biotech and Pharma Employers survey.

As global demand for food and fuel continues to rise, we are dedicated to our purpose: Bringing plant potential to life. Syngenta is one of the world's leading companies with more than 26,000 employees in over 90 countries. We work in a collaborative and inspiring culture where personal contribution is rewarded and growth and development are at the heart of our culture.

Through our world-class science, global reach and commitment to working with our customers, we help to increase crop quality and productivity, protect the environment and improve health and quality of life.

There's never been a more important time to join Syngenta. Visit www.syngenta.com



Massachusetts
Institute of
Technology

Come work with us!

Assistant Professor The McGovern Institute for Brain Research

The McGovern Institute's general focus is in systems neuroscience with an emphasis on the neural basis of perception, cognition, and action. We are seeking a candidate with a research focus in any of these three areas, using human subjects and/or computational methods. We would regard it as a plus if the candidate's work bridges levels using a variety of tools and/or the candidate were interested in translating basic research findings into new ideas for studying the pathophysiology or treatment of brain disorders. Successful applicants are expected to develop and lead independent, internationally competitive research programs and to share our commitment to undergraduate and graduate education by teaching courses and supervising graduate and undergraduate research.

The mission of the McGovern Institute is to understand the relationship of neuronal processes, circuits and computations to behavior, ultimately providing benefits to human health and welfare. Research in the McGovern Institute is expected to help people with brain disorders ranging from sensory system impairments to movement disorders and emotional and cognitive disorders. For more information on the McGovern Institute please visit our website at <http://mcgovern.mit.edu>

Applicants should submit a curriculum vitae, a summary of current and proposed research programs, a publication list, and should arrange for three letters of recommendation to be sent electronically via Academic Jobs Online (<https://academicjobsonline.org/ajob/jobs/1884>). Consideration of applications will begin on November 1, 2012 and will continue until the position is filled.

MIT is an affirmative action employer,
and we encourage applications from
women and underrepresented minorities.

McGOVERN INSTITUTE
FOR BRAIN RESEARCH AT MIT

<http://web.mit.edu>



FACULTY POSITIONS IN SYNTHETIC ORGANIC CHEMISTRY, CELLULAR/ MOLECULAR/MICROBIOLOGY, and BIOMEDICAL ENGINEERING

The Department of Chemistry, Chemical Biology, and Biomedical Engineering at the **Stevens Institute of Technology** invites applications for three **tenure-track** positions at the level of **Assistant or Associate Professor** rank in the areas of (1) synthetic organic chemistry, (2) cellular, molecular, or microbiology, and (3) biomedical engineering with an emphasis in electrical engineering. The successful applicants will hold a PhD with postdoctoral experience and a good publication record. It is our expectation that the successful candidates will develop dynamic, externally-funded research programs and will teach undergraduate and graduate courses. We are particularly interested in attracting candidates with research interests that complement our existing institutional strengths in development and delivery of novel therapeutic agents and the engineering/physiology interfaces involved in biorobotics and biomaterials. Our undergraduate and graduate programs in Chemistry and Chemical Biology and in Biomedical Engineering enjoy interactions with New Jersey and New York City metropolitan area academic, healthcare, and pharma/biotech institutions and host over 300 highly motivated and talented undergraduate and graduate students. The Stevens Institute of Technology has a 140-year history of advancing engineering, science, and technology.

For full consideration, please submit a curriculum vitae, research plan, teaching statement, and three letters of reference to **Dr. Philip L. Leopold, Stevens Institute of Technology, CCBBME, Castle Point on Hudson, Hoboken, NJ 07030** (email: pleopold@stevens.edu) by **November 15, 2012**.

*The Stevens Institute of Technology is an Equal Opportunity/
Affirmative Action Employer.*



ILLINOIS

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

David R. and Margaret Stirewalt Lincicome Professor of Host-Parasite Interactions Department of Animal Biology School of Integrative Biology

The Department of Animal Biology and the School of Integrative Biology at the University of Illinois, Urbana-Champaign seek a highly qualified candidate for the **David R. and Margaret Stirewalt Lincicome Professor of Host-Parasite Interactions**. This is a full-time faculty position at the rank of **Associate or Full Professor** with credentials warranting tenure at the University of Illinois. We seek a broadly trained biologist who has a well-established, internationally renowned, externally funded research program in any aspect of host-parasite interactions, including but not limited to coevolutionary interactions, the molecular, physiological, developmental, or immunological bases of such interactions, effects on host behavior, life histories, population dynamics, conservation biology, or alterations in such interactions caused by global change. We welcome empirical and theoretical approaches. The successful candidate will have the opportunity to be part of dynamic and well-established communities of integrative biologists with interests spanning a wide range of taxa in the School of Integrative Biology, as well as in a number of interdisciplinary programs across the campus. Responsibilities also include teaching and participation in both undergraduate and graduate training. The successful candidate must have a Ph.D. in biology or related discipline. Salary is commensurate with qualifications and experience. Target start date is August 16, 2013 but is negotiable.

To ensure full consideration, please create your candidate profile through <http://go.illinois.edu/LincicomeProf> and upload your application letter, curriculum vitae, summary of research and plans, teaching philosophy and experience, and contact information for three professional references by **November 16, 2012**. After a review of the research record, the search committee may then contact the applicant about soliciting letters of reference. Applicants may be interviewed before the closing date; however, no hiring decision will be made until after that date. For further information contact Host-Parasite Interactions Search Chair, sib@life.illinois.edu.

Illinois is an Affirmative Action/Equal Opportunity Employer and welcomes individuals with diverse backgrounds, experiences, and ideas who embrace and value diversity and inclusivity. (www.inclusiveillinois.illinois.edu).



UNIVERSITY AT ALBANY
State University of New York

Two Open Rank Positions at The RNA Institute

CRYSTALLOGRAPHER conducting research on RNA and its functional complexes with nucleic acids, proteins, small molecules, etc.

COMPUTATIONAL CHEMIST/BIOINFORMATICIAN conducting research on structure, function, and dynamics of RNA and its functional complexes through both computational and wet-lab approaches.

Positions entail a competitive salary, start-up package, and research space in the newly constructed RNA Institute (<http://www.albany.edu/rna>), with access to state-of-the-art facilities housed in the Life Sciences Research Building (<http://www.albany.edu/lifesciences>).

Qualifications: Ph.D. from a university accredited by the U.S. Department of Education or by an internationally recognized accrediting organization. At the Assistant Professor level, preferred candidates will have postdoctoral training and show promise as independent, extramurally funded investigators. At the Associate and Professor levels, preferred candidates will have a significant trajectory of publications, external funding and mentoring.

Application Process: Applicants should submit to the appropriate website a CV with publications; past, present, and/or pending funding; statements of research and teaching interests:

CRYSTALLOGRAPHER:

<http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=34153>

COMPUTATIONAL CHEMIST/BIOINFORMATICIAN:

<http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=34150>

Applicants must address their ability to work with and instruct a culturally diverse population. They must arrange for three or more reference letters to be sent on their behalf directly to rnasearch@albany.edu. Review of complete applications will commence on October 15th and continue until the positions are filled. Incomplete applications will not be considered.

The RNA Institute creates an outstanding environment for research collaborations by bringing together ~40 principal investigators from within UAlbany and regional institutions (<http://www.albany.edu/rna>).

The University at Albany is an EEO/AA/IRCA/ADA employer.

UT SOUTHWESTERN MEDICAL CENTER AT DALLAS

ENDOWED SCHOLARS PROGRAM IN MEDICAL SCIENCE

Unique and highly competitive, **THE ENDOWED SCHOLARS PROGRAM IN MEDICAL SCIENCE** is designed to launch the next generation's scientific leaders on their biomedical research careers by providing seed money, research space and startup support for groundbreaking projects. The Endowed Scholars Program gives early-career investigators the chance to take risks, supported by the mentoring of experienced and highly distinguished established researchers at **The University of Texas Southwestern Medical Center at Dallas**.

As one of the foremost research institutions in the world, UT Southwestern, with four Nobel Prizes awarded to its faculty since 1985 and 18 members of the National Academy of Sciences, is poised to lead the way in a new era of scientific discovery in the 21st century. We conduct more than 3,500 research projects annually totaling more than \$400 million. Endowed Scholars have access to exceptional core facilities, which include DNA microarray services; electron microscopy; live-cell imaging; mouse gene knockouts, transgenesis and metabolic phenotyping; high-throughput chemical screening; and structural biology. UT Southwestern also is home to one of the nation's first 7-Tesla magnetic resonance imaging devices for human studies. UT Southwestern has a vibrant graduate school and nearly 4,400 medical, graduate and health professions students, residents and postdoctoral fellows.

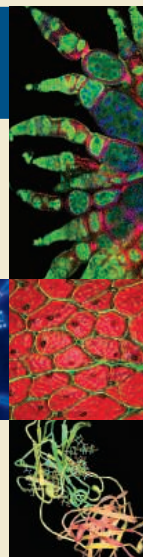
The Endowed Scholars Program, which is fully funded from private endowment, provides more than \$1,000,000 over four years to support the independent research activities, salary and benefits of each scholar. Up to five new scholars are selected each year from top universities, institutions and laboratories around the world. Each scholar is appointed as a tenure-track assistant professor in a UT Southwestern academic department or research center. Positions in both basic science and clinical departments are available.

Potential scholars submit nomination materials to a chair or director of one of UT Southwestern's basic or clinical academic departments or research centers. Those chairs or directors then forward finalists' materials for consideration to the medical center's Endowed Scholars Committee.

For detailed information about nomination materials and currently available positions, please visit our Web page: utsouthwestern.edu/utsw/home/scholars

*Applications from women and underrepresented minority candidates are strongly encouraged.
UT Southwestern is an equal opportunity institution.*

UT SOUTHWESTERN
MEDICAL CENTER



THE INSTITUTE FOR MOLECULAR ENGINEERING

New Faculty for a New Field

The University of Chicago has launched a unique, interdisciplinary Institute for Molecular Engineering (IME) with the aim of assembling a faculty to address some of the biggest technological challenges facing humanity in energy, environmental stewardship, health care, biotechnology, information, and communications. To tackle these problems and educate a new generation of engineers, the IME will hire faculty in areas spanning a range of expertise pertinent to engineering at the molecular level. An independent unit within the University, IME is also formally affiliated with Argonne National Laboratory. Joint appointments with Argonne or other UChicago units will be encouraged where appropriate.

Important areas for initial hires include:

- Materials synthesis, including organic, inorganic, and semiconductor materials
- Device fabrication and assembly at the micro- and nano-scales
- Quantum information engineering
- Imaging and other structural determination tools
- Biological and biomedical engineering, including synthetic and systems biology, bio-inspired materials, and regenerative medicine
- Medical diagnostics and therapeutics
- Computational engineering, including multi-scale modeling and prediction

Learn more at molecularengineering.uchicago.edu/science

The University of Chicago is an Equal Opportunity/Affirmative Action Employer.



Assembling Faculty Leaders

Along with founding Pritzker Director **Matthew Tirrell**, these new faculty members in the IME, each of whom holds a joint appointment at Argonne National Laboratory, will provide senior leadership for the institute's research mission of exploring innovative technologies in nanoscale manipulation and design at a molecular scale.



DAVID AWSCHALOM

Liew Family Professor in Molecular Engineering

Currently a professor of physics and electrical and computer engineering at UC Santa Barbara, Awschalom is one of the world's leaders in spintronics and quantum information engineering.



JUAN DE PABLO

Liew Family Professor in Molecular Engineering
Previously the Howard Curler Distinguished Professor and Hilldale

Professor of Chemical Engineering at University of Wisconsin-Madison, de Pablo conducts supercomputer simulations to understand and design new materials and find applications for them.



PAUL NEALEY

Brady W. Dougan Professor in Molecular Engineering

Previously the Shoemaker Professor of Chemical and Biological Engineering at UW-Madison, Nealey is a pioneer in directed self-assembly and one of the world's leading experts on patterning organic materials.



FACULTY POSITION IN THE DEPARTMENT OF MATERIALS SCIENCE AND ENGINEERING AT DREXEL UNIVERSITY

The Department of Materials Science & Engineering at Drexel University (www.mse.drexel.edu) is seeking applications for a tenured/tenure-track faculty position. Of particular interest are candidates with a demonstrated record of excellence in original research in either:

- (1) soft and hybrid material design, synthesis, assembly, and function or
- (2) electrochemistry with emphasis in energy storage.

A Ph.D. in Materials Science and Engineering or a closely related field is preferred.

The ideal applicant should also possess research interests in an emerging research area such as advanced energy technologies, biomedical materials and devices, or environmental sustainability, and be able to contribute to building interdisciplinary, integrated research program focused in their area of expertise.

Applicants should submit a cover letter; a full curriculum vitae; statements of research and teaching plans; and names and contact information of three references online at <http://www.materials.drexel.edu/faculty/positions/>. If a female candidate is selected at the Assistant Professor level, she will be eligible for the Anne Stevens Assistant Professorship. The position is available immediately and applications will be considered until the position is filled.

Drexel University is an Equal Opportunity Employer and encourages applications from qualified women and minorities.



TEXAS TECH UNIVERSITY

Structural Biology: Biological NMR Spectroscopy

The Department of Chemistry and Biochemistry invites applications for a senior faculty member in the area of **Biological NMR Spectroscopy**. We are especially interested in applicants whose research programs will complement existing areas of strength at TTU, such as structure, function and dynamics of membrane proteins, biomolecules involved in cancer, and plant proteins. However, all qualified candidates are encouraged to apply. The successful candidate for this position will be part of a major new **Structural Biology** initiative at TTU that includes a commitment to the acquisition of 600 MHz and 800 MHz NMR instruments and hiring two additional biological NMR spectroscopy faculty. Evidence of a well-funded research program and a demonstrated commitment to excellence in teaching and service are essential. The successful applicant will be directly involved in the selection of the NMR equipment and in the selection of the two additional faculty to be hired at the assistant and/or associate professor level. The Department of Chemistry and Biochemistry is among the top academic units at Texas Tech University, in terms of research funding, publications and graduate education. Texas Tech University is classified as a doctoral research-intensive university by the Carnegie Foundation; it has an enrollment of more than 30,000 students, and is one of the major, state-supported, multidisciplinary universities of the Southwest. A School of Medicine is located on the main campus in Lubbock.

All applications must be submitted online. Online faculty application for requisition number 85807 can be found at <http://jobs.texasstate.edu>. Applications must include the names of three references. Evaluation of applications will continue until the position is filled. For additional information, please contact the chair of the search committee, **Dr. Joachim Weber, Department of Chemistry and Biochemistry, Texas Tech University, Box 41061, Lubbock, TX 79409-1061** (joachim.weber@ttu.edu).

Texas Tech University is an Affirmative Action/Equal Opportunity Employer, committed to excellence through diversity. Texas Tech welcomes applications from minorities, women, veterans, persons with disabilities, dual-career couples, and all qualified persons.



Tenure-track Assistant or Associate Professor of Genetics/Genome Sciences

The Department of Human Genetics at the University of Utah School of Medicine (www.genetics.utah.edu) seeks outstanding applicants for tenure-track positions at the level of Assistant or Associate Professor. We are looking for highly creative scientists who use genetics to investigate complex biological problems. We especially encourage applicants whose research focuses on human and medical genetics using functional, computational, or evolutionary genomics and/or model organisms. As part of a vibrant community of collaborative faculty on campus, the Department of Human Genetics lies at the interface between basic and clinical sciences, creating ample opportunities for interdisciplinary studies. Our institution is set in a unique recreational and geographical landscape that attracts a very diverse and productive scientific community. Successful candidates will receive a generous startup package and enjoy a stimulating research environment that places a strong emphasis on innovation and interaction.

To apply for this position, please see the following website: <http://utah.peopleadmin.com/postings/18655>. For questions regarding this posting or your application, please contact Natalie Johnson at: njohnson@genetics.utah.edu.

The University of Utah is an Equal Opportunity/Affirmative Action Employer and Educator. Minorities, women, and persons with disabilities are strongly encouraged to apply. Veterans preference. Reasonable accommodations provided. For additional information: <http://www.regulations.utah.edu/humanResources/5-106.html>.

The University of Utah values candidates who have experience working in settings with students from diverse backgrounds, and possess a [strong or demonstrated] commitment to improving access to higher education for historically underrepresented students.

The University of Utah Health Sciences Center is a patient focused center distinguished by collaboration, excellence, leadership, and Respect. The University of Utah HSC values candidates who are committed to fostering and furthering the culture of compassion, collaboration, innovation, accountability, diversity, integrity, quality, and trust that is integral to the mission of the University of Utah Health Sciences Center.

LET'S SCALE UP THE POSSIBILITIES

Researcher Biodomain (Graduate/Entry Level), Houston, TX

Do you have a PhD in microbiology/biochemistry with a specific knowledge of larger scale microbe (bacteria and yeast) fermentation? Then we'd like you to help us pilot an exciting new project.

Joining our in-house fermentation team, you'll scale up our current lab-sized fermentation of different biofuel components to plant scale. You'll need experience in scaling up yeast and/or bacterial fermentation and product extraction at the 10+litre scale (and, preferably, 100+ litre).

If you have the ability to think big and want to join a team of trusted and supportive colleagues, apply online at www.shell.us/phdcareers.

To be considered for this full-time graduate role, you must apply for a Shell Recruitment Day.

Let's deliver better energy solutions together.



An Affirmative Action / Equal Opportunity Employer, M/F/D/V.



Faculty Position in Stem Cell Biology

The Children's Research Institute (CRI) at the University of Texas-Southwestern Medical Center in Dallas, TX seeks applications for a **tenure-track faculty position in the area of stem cell biology**. Outstanding investigators at any rank will be considered. Candidates must have a Ph.D., M.D. or equivalent degrees and the ability to direct an independently-funded research program exploring any aspect of stem cell biology.

The UT-Southwestern Medical Center has a long and distinguished history of excellence in disease-related basic science research. The CRI is a new institute recruiting outstanding individuals dedicated to solving fundamental problems in biology and disease. The CRI is a dynamic, stimulating, and highly collaborative scientific environment. Major areas of focus within the CRI will include stem cell biology, cancer biology, and metabolism.

Please submit a CV, a 2-page summary of past accomplishments and research plans, and ask three references to submit letters by **November 1, 2012** to CRIApplicants@utsouthwestern.edu.

UT Southwestern is an Equal Opportunity/Affirmative Action Employer.



GEISEL
SCHOOL OF
MEDICINE
AT DARTMOUTH

Professor of Biostatistics Geisel School of Medicine at Dartmouth

The Geisel School of Medicine at Dartmouth is seeking a senior **Biostatistician, at the rank of Associate or Full Professor**, to conduct innovative methodological and collaborative research, mentor faculty, and teach biostatistics within the Norris-Cotton Cancer Center and other academic units throughout the Geisel School. Under the newly named Geisel School, there is strengthened focus, and expanding faculty, in Biostatistics at Dartmouth under the leadership of Dr. Chris Amos. Additionally, an innovative new Quantitative Biomedical Sciences graduate program commenced in the fall 2011 as the first of its kind in the country. There are currently eight full-time, and several part-time, statistical faculty members at the Geisel School, along with twenty-two master's levels statistical analysts who are engaged actively in extramurally-funded statistical and biomedical research. Current interests of faculty in statistical methods include: clinical trials, measurement error methods, biomedical imaging, genomics and molecular epidemiology, survival analysis, multivariate models, spatial statistics in epidemiology, semi-parametric methods, longitudinal methods, diagnostic testing, optimal epidemiologic design, and prevention and therapeutic trials.

Founded in 1797, the Geisel School of Medicine at Dartmouth draws on the resources of Dartmouth College and Dartmouth-Hitchcock Medical Center for broad interdisciplinary programs in biomedical research, education, patient care and service. Located in rural New Hampshire, the region uniquely offers idyllic landscapes and recreation, along with outstanding schools and cultural activities.

Applicants should have a doctoral degree in biostatistics or statistics and have nationally and/or internationally recognized contributions to statistical methods, statistical education, and multi-disciplinary collaborative research. In addition to significant experience in biostatistics, preferably with a translational or molecular-genetic focus, applicants should possess a track record of NIH research funding. Submissions should include a letter of intent, curriculum vitae, and names and contact information for three references. Review of applications will commence immediately and will continue until positions are filled.

Salary Range: Competitive

Benefits: Outstanding benefits package and faculty development support.

Applicant materials should be e-mailed or mailed to: **Biostatistics Professor Search, The Geisel School of Medicine at Dartmouth, Attention: Crystal Flaherty, One Medical Center Drive, 7927 Rubin Building, Lebanon, New Hampshire 03756; Crystal.Flaherty@Dartmouth.edu**. For more information on the Section of Biostatistics and Epidemiology: <http://bio-epi.hitchcock.org>. For more information on the Quantitative Biomedical Sciences graduate program: <http://iqbs.org/>.

The Geisel School of Medicine at Dartmouth is an Equal Opportunity and Affirmative Action Employer. We welcome applications from, and will extend equal opportunity to, all individuals without regard for gender, race, religion, color, national origin, sexual orientation, age, disability, handicap or veteran status.



ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Assistant, Associate or Full Professor of Quantitative Ecology School of Integrative Biology

The School of Integrative Biology and the Department of Plant Biology at the University of Illinois, Urbana-Champaign invite applications for a full-time, nine-month, tenure-track or tenured faculty position in integrative plant biology at the rank of **Assistant, Associate or Full Professor**. Target start date is 16 August 2013. We seek an outstanding ecologist who uses mathematical, statistical, numerical-modeling, or theoretical approaches to study ecosystem dynamics at community to global scales. Research areas of focus include, but are not limited to, ecological forecasting, biosphere-atmosphere interactions, and climate change. The successful candidate will be expected to develop an externally funded research program, teach at undergraduate and graduate levels, and collaborate with faculty to develop research and education initiatives in quantitative ecology and mathematical biology. She/he will have the opportunity to be part of dynamic and well-established communities of integrative biologists with interests spanning a wide range of taxa in the School of Integrative Biology, as well as in a number of interdisciplinary programs across the campus. A Ph.D. or equivalent is required. Postdoctoral experience is desirable for candidates at the assistant professor level. Associate and full professor candidates should present an established record of scholarly publications and demonstrate excellence in teaching. Salary and rank are commensurate with qualifications and experience.

To ensure full consideration, please create your candidate profile through <http://go.illinois.edu/QuantitativeEcology> and upload your application letter, curriculum vitae, summary of research and plans, teaching philosophy and experience, and contact information for three professional references by 21 October 2012. After a review of the research record, the search committee may then contact the applicant about soliciting letters of reference.

Applicants may be interviewed before the closing date; however, no hiring decision will be made until after that date. For further information contact Quantitative Ecology Search Chair, sib@life.illinois.edu.

Illinois is an Affirmative Action /Equal Opportunity Employer and welcomes individuals with diverse backgrounds, experiences, and ideas who embrace and value diversity and inclusivity. (www.inclusiveillinois.illinois.edu).



TEXAS A&M
UNIVERSITY

Assistant Professor Tenure-track

Applications are invited for a tenure-track **Assistant Professor** position in either the **Department of Biochemistry and Biophysics** or the **Department of Plant Pathology and Microbiology**, depending on the credentials and professional goals of the successful candidate. This search will fill the fourth position authorized for expansion of the **Center for Phage Technology (CPT)**, an interdisciplinary center established by the Texas A&M University System Board of Regents in 2010 and focused on translating basic science into emerging bacteriophage technologies. However, the search is not restricted to scientists with direct involvement in phage research. We seek an exceptional scientist committed to establishing a nationally-competitive research program. There is heightened interest for applicants with expertise synergistic with understanding phage biology and its applications, particularly in the areas of synthetic biology, biophysics, structural biology, and bacteria-plant interactions. Nevertheless, the excellence of the individual candidate will take precedence over the area of special interest. Competitive salary, start-up funds, laboratory space and access to instrumental cores will be provided. The successful candidate will have a doctoral degree in a relevant field and be expected to teach courses in relevant disciplines.

Please send a PDF file that contains a cover letter, CV, research summary (past, present, and planned), a teaching statement and contact information for three professional references to: cpt@tamu.edu. Applications received by **October 30, 2012** will be guaranteed full consideration by the Search Committee, although review of applications will continue until the position is filled.

Equal Opportunity Employer.

TEXAS A&M
AGRI LIFE
RESEARCH

POSITIONS OPEN



FACULTY POSITION in Molecular Parasitology

The Department of Pharmacology at The University of Texas (U.T.) Southwestern Medical Center invites applications for tenure-track faculty positions at the level of **ASSISTANT PROFESSOR** in molecular parasitology, with a research emphasis on protozoal pathogens. We particularly encourage applications from those with backgrounds in studying the biology of the malaria, trypanosome (African and South American), leishmania and giardia parasites. The Department is strongly research-oriented with major programs in Cell Signaling, Nuclear receptor signaling, Cell Cycle Regulation, Nuclear and Vesicular Trafficking, Cancer Biology, Chemical Biology and Parasite Biology. Parasite Biology is a growing interest within the department, and faculty within the department have established programs working on both protozoal and worm pathogens. Scientists within the Department participate in a vibrant, interdepartmental, and highly collaborative research community within the University and enjoy unparalleled access to state-of-the-art research cores in cellular imaging, protein chemistry, high throughput screening, animal husbandry and metabolic phenotyping, X-ray crystallography and NMR spectroscopy.

Competitive applicants must have a relevant Ph.D. or M.D. degree, postdoctoral training, and show evidence of firm commitment to an independent research career. Outstanding candidates will be eligible for a position in the Endowed Scholar program ([website: http://www.utsouthwestern.edu/research/programs/endowed-scholars-program.html](http://www.utsouthwestern.edu/research/programs/endowed-scholars-program.html)).

Please submit curriculum vitae, a concise description of research plans (three-page limit) and three letters of reference by November 15, 2012 to e-mail: rxfacearch@utsouthwestern.edu. Attention: Meg Phillips, Chair, Pharmacology Faculty Search Committee, U.T. Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-8816.

The University of Texas Southwestern Medical Center is an Affirmative Action/Equal Opportunity Employer. Women and minority candidates are encouraged to apply.

DIRECTOR OF BIOINFORMATICS

Program in Molecular Medicine

University of Massachusetts Medical School

The Program in Molecular Medicine seeks an outstanding individual to provide bioinformatics support and collaboration to its faculty research laboratories. The Program consists of basic scientists and physician scientists representing a broad range of biomedical disciplines, and operates as an academic department in the Medical School ([website: http://umassmed.edu/pmm/](http://umassmed.edu/pmm/)). The Director of Bioinformatics will have a terrific opportunity to participate in multiple biomedical research projects by applying bioinformatics skills to optimally leverage state-of-the-art technologies, such as next-generation sequencing, genotyping, proteomics, and imaging.

Candidates must have a Ph.D. and at least three years additional experience in applying bioinformatics to biomedical research.

Applicants should submit a cover letter explaining their qualifications and interest in this position, curriculum vitae, and contact information for three reference letters to [website: http://www.academicjobsonline.org](http://www.academicjobsonline.org): #1977. Inquiries, but not application materials, may be addressed to e-mail: john.sullivan@umassmed.edu.

Review of applications will continue until the position is filled. As an Equal Opportunity and Affirmative Action Employer, the University of Massachusetts Medical School recognizes the power of a diverse community and encourages applications from individuals with varied experiences, perspectives, and backgrounds. Minorities and women are especially encouraged to apply.

POSITIONS OPEN



FACULTY POSITION in Pharmacology

The Department of Pharmacology at The University of Texas (U.T.) Southwestern Medical Center invites applications for a tenure-track faculty position at the level of **ASSISTANT PROFESSOR**. We are particularly interested in strong candidates who wish to apply molecular mechanisms of biological processes, both in vitro and in vivo, to study various diseases, including cancer, obesity and diabetes, and parasitism. U.T. Southwestern provides a highly collaborative research community with unparalleled access to state-of-the-art research cores in cellular imaging, high throughput screening, animal husbandry and metabolic phenotyping, X-ray crystallography and NMR spectroscopy, and protein and small molecule mass spectrometry.

Competitive applicants must have a relevant Ph.D. or M.D. degree, postdoctoral training, and show evidence of firm commitment to an independent research career. Outstanding candidates will be eligible for a position in the Endowed Scholar program ([website: http://www.utsouthwestern.edu/research/programs/endowed-scholars-program.html](http://www.utsouthwestern.edu/research/programs/endowed-scholars-program.html)) or as a Cancer Preventive Research Institute of Texas (CPRIT) Scholar in Cancer Research ([website: http://www.cprit.state.tx.us/images/uploads/rfa_r-12-rft-11.pdf](http://www.cprit.state.tx.us/images/uploads/rfa_r-12-rft-11.pdf)).

Please submit curriculum vitae, a concise description of research plans (three-page limit), and three letters of reference by November 15, 2012 to e-mail: rxfacearch@utsouthwestern.edu. Attention: Meg Phillips, Chair, Pharmacology Faculty Search Committee, U.T. Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-8816.

The University of Texas Southwestern Medical Center is an Affirmative Action/Equal Opportunity Employer. Women and minority candidates are encouraged to apply.

MEDICAL ENTOMOLOGIST

Assistant Professor

University of Florida Florida

Medical Entomology Laboratory

Institute of Food and Agricultural Sciences

The Florida Medical Entomology Laboratory (FMEI) invites applications for a tenure-track faculty position at the **ASSISTANT PROFESSOR** level from outstanding scientists interested in the biology and ecology of blood-feeding arthropods and the pathogens they transmit. Experience in aspects of medical entomology including a knowledge of techniques used in other disciplines to analyze and interpret ecology, epidemiology, and the vector-pathogen-host interface is essential. Applicants should visit [website: http://jobs.ufl.edu/postings/31049](http://jobs.ufl.edu/postings/31049) for detailed information about the position and the FMEI [website: http://fmel.ifas.ufl.edu](http://fmel.ifas.ufl.edu) to learn more about the laboratory. Review of applications will begin November 30, 2012 and continue until the position is filled.

The University of Florida is an Equal Opportunity Institution dedicated to building a broadly diverse and inclusive faculty and staff.

DIRECTOR, CHEMICAL AND

BIOPHYSICAL

Instrumentation Center

The Department of Chemistry at Yale University, New Haven, CT invites applications for the position of Director, Chemical and Biophysical Instrumentation Center to commence January 1, 2013. Reporting to the Lead Administrator for Chemistry and the Chair of the Instrument Committee, the Director is responsible for the daily operations, staff oversight, planning, and financial management of the Chemical and Biophysical Instrumentation Center (CBIC), which encompasses a suite of NMR, X-ray crystallography, chemical and biophysical instrumentation. Applicants should apply online at [website: http://www.yale.edu/hronline/careers/application/external/index.html](http://www.yale.edu/hronline/careers/application/external/index.html). Requisition #18688BR.

Yale University is an Equal Opportunity/Affirmative Action Employer and applications from women and underrepresented minority group members are especially encouraged.

POSITIONS OPEN



FACULTY POSITION in Ecological and Evolutionary Physiology Department of Biological Sciences

The Department of Biological Sciences at Dartmouth seeks applicants for a tenure-track **ASSISTANT PROFESSORSHIP** in physiology. We seek candidates who study physiological processes at the biochemical, cellular or whole organism level and who are using the tools of biochemistry, biophysics, engineering, mathematics, or computer modeling with the goal of understanding their evolutionary or ecological significance. Areas of interest include the physiology of adaptation, functional morphology, biomechanics, or comparative analyses of morphological, physiological, and functional diversity. Candidates who are taking empirical, theoretical, or statistical approaches to major questions in these areas in any biological system are welcome. The successful candidate will be expected to supervise an independent research program that will attract extramural funding, to provide research training for graduate and undergraduate students, and to teach physiology at the undergraduate and graduate levels. Application materials should include curriculum vitae, representative publications, statements of research and teaching interests, and the names of at least three references. Please send materials electronically to e-mail: physiologist.search@cloud.dartmouth.edu.

Application review will begin on October 10, 2012 and continue until the position is filled. For further information about the department and graduate programs, see [website: http://www.dartmouth.edu/~biology/](http://www.dartmouth.edu/~biology/).

Dartmouth is an Equal Opportunity and Affirmative Action Employer. We welcome applications from & will extend equal opportunity to all individuals without regard for gender, race, religion, color, national origin, sexual orientation, age, disability, handicap, or veteran status.

TENURE-TRACK POSITION

Department of Chemical Engineering and Materials Science

University of California, Davis

Applications are invited for a faculty position at the **ASSISTANT PROFESSOR** level in chemical engineering. All areas of expertise will be considered, and candidates with an interest in the area of catalysis are especially encouraged to apply. The candidate should have a strong research record with the potential and commitment to become a leader in the field. Commitment to undergraduate and graduate education is essential. A Ph.D. in chemical engineering or a related discipline is required. Consult [website: http://chms.engineering.ucdavis.edu/](http://chms.engineering.ucdavis.edu/) for our on-line application procedure and requirements. The position is open until filled; but to assure full consideration, applications should be submitted no later than October 19, 2012, for a start date of July 1, 2013.

UC Davis is an Affirmative Action/Equal Opportunity Employer, and is dedicated to recruiting a diverse faculty community. We welcome all qualified applicants to apply, including women, minorities, individuals with disabilities, and veterans.

MIDDLE TENNESSEE STATE UNIVERSITY

The Department of Biology at Middle Tennessee State University invites applications for two **ASSISTANT/ASSOCIATE PROFESSOR** positions: **CELL BIOLOGIST** and **MICROBIOLOGIST**. Application materials must be filed online at [website: http://mtsujobs.mtsu.edu](http://mtsujobs.mtsu.edu) and should include: cover letter, employment application, curriculum vitae, statement of research interests, and statement of teaching philosophy. See [website: http://www.mtsu.edu/biology/](http://www.mtsu.edu/biology/) for more information about the department. Review of applications begins October 29, 2012. Equal Opportunity/Affirmative Action Employer.



The University of Georgia

Assistant or Associate Professor in Pharmaceutical and Biomedical Sciences

The Department of Pharmaceutical and Biomedical Sciences (<http://pbs.rx.uga.edu>) in the College of Pharmacy at the University of Georgia invites applications for a tenure-track position at the Assistant or Associate Professor level with a particular interest in individuals who employ chemical, biochemical, molecular or pharmaceutical techniques to develop new drugs, study drug functions and metabolism, or improve treatments for diseases.

The Department offers a unique multidisciplinary environment with strengths in medicinal chemistry, biochemistry, pharmacology, toxicology, pharmacokinetics, analytical chemistry, cancer and neurosciences. Additional resources include new state-of-the-art facilities, a generous start-up package and a strong graduate program.

Candidates are required to have a PhD, MD or equivalent in Pharmaceutical Sciences, Pharmacology, Synthetic Chemistry, Biochemistry, Molecular Biology, Cell Biology, Neuroscience or related disciplines and a strong record of publications in peer-reviewed scientific journals. Successful candidates are expected to establish and maintain a strong extramural-funded research program. Commitment to excellence in teaching at the undergraduate and graduate levels is also required. Preference will be given to candidates with a currently funded research program.

To apply, submit a cover letter, curriculum vitae and a summary of research interests in a single PDF document to pbsearch@uga.edu. Please refer to **position #67435**. In addition, applicants should arrange to have three letters of reference submitted electronically to the same e-mail address. The position will remain open until filled.

The University of Georgia is an Equal Opportunity/Affirmative Action Employer. Women, Minorities, Veterans and Persons with Disabilities are encouraged to apply.

Faculty Position in the Gene Expression and Regulation Program at The Wistar Institute

The Wistar Institute, an independent, non-profit research institute with a primary focus on cancer research, is seeking an outstanding candidate with a doctoral degree or equivalent for a faculty position at the Assistant or Associate Professor level in the Gene Expression and Regulation Program. We are seeking a candidate to complement the Institute's strength in epigenetic mechanisms in chromatin and transcription regulation. The successful candidate will use chemical, biochemical or genomic approaches within the biological context of diseases such as cancer and other age-associated disorders. Specific areas of interest include chromatin regulatory mechanisms, chemical biology, enzymology, structure/biophysics of higher-order chromatin, biological models of chromatin regulation, nuclear organization of transcription, and non-coding RNA regulatory mechanisms.

The Wistar Institute, an NCI-designated Cancer Center, offers highly competitive start-up support, salary and fringe benefits in addition to a superb and interactive research environment, including state-of-the-art core facilities with multiple laboratories that are increasingly emphasizing systems biology approaches to study basic biological processes, cancer and other human diseases. The Institute's location on the University of Pennsylvania campus provides potential academic and clinical collaborators, as well as opportunities for training graduate students.

Applications will be reviewed as received and will be accepted until the position is filled. To ensure timely consideration, applicants should submit applications before October 31, 2012. The application should include a curriculum vitae, a brief summary of past and future research interests, history of research funding support (if applicable), and three letters of reference. Applications should be sent by e-mail to: **Ronen Marmorstein, Search Committee Chair, c/o Maria Colelli (coelli@wistar.org), The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104. EOE/AA/M/F/D/V.**



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TENURE TRACK FACULTY POSITIONS Physiology

The Department of Physiology at Wayne State University (WSU) School of Medicine invites applications for tenure-track Assistant/Associate Professor positions. Major areas of interest include cellular, molecular, and systems approaches to questions related to pathophysiology and developmental biology. Special consideration will be given to investigators who complement existing strengths of the Department (<http://physiology.med.wayne.edu>). One position will be offered jointly with the Department of Obstetrics and Gynecology as part of a research and graduate education program in Reproductive Sciences, based at the C.S. Mott Center for Human Growth and Development (<http://mott.med.wayne.edu>). For this joint position preference will be given to an individual with an active translational stem cell program.

Candidates are expected to establish active extramurally funded research programs and participate in teaching medical/graduate students. Start-up packages and salaries are highly competitive. Candidates must hold Ph.D. or M.D. or equivalent and apply with curriculum vitae, detailed research plan and names/contact information of three references to WSUPhysiologyFacultySearch@med.wayne.edu. Review of applications will begin after **November 1, 2012** and continue until positions are filled.

WSU offers 350 academic programs through 14 schools and colleges to over 31,000 students in the Detroit Midtown area. WSU School of Medicine is a state-of-the-art research environment, and rated by the Carnegie Foundation in the top third of all U.S. Research Institutions. The Detroit Metro area is home to four million people and a hub to many of the nation's high-tech industries.

WSU is an Equal Opportunity/Affirmative Action Employer.



Faculty Position in Cancer Biology

The Children's Research Institute (CRI) at the University of Texas-Southwestern Medical Center in Dallas, TX seeks applications for a **tenure-track faculty position in the area of cancer biology**. Outstanding investigators at any rank will be considered. Candidates must have a Ph.D., M.D. or equivalent degrees and the ability to direct an independently-funded research program exploring any aspect of cancer biology.

The UT-Southwestern Medical Center has a long and distinguished history of excellence in disease-related basic science research. The CRI is a new institute recruiting outstanding individuals dedicated to solving fundamental problems in biology and disease. The CRI is a dynamic, stimulating, and highly collaborative scientific environment. Major areas of focus within the CRI will include stem cell biology, cancer biology, and metabolism.

Please submit a CV, a 2-page summary of past accomplishments and research plans, and ask three references to submit letters by **November 1, 2012** to CRIApplicants@utsouthwestern.edu.

UT Southwestern is an Equal Opportunity/Affirmative Action Employer.

POSITIONS OPEN



FACULTY POSITION – MICROBIOLOGY University of Connecticut - Storrs

The Department of Molecular and Cell Biology at the University of Connecticut seeks applicants for a nine-month **ASSISTANT PROFESSOR** tenure-track position in Microbiology, starting August 23, 2013 (search #2013172). We are particularly interested in candidates working on microbiomes, host-microbe interactions or the ecology and evolution of microbial communities. For details on the position, qualifications, and application instructions please visit **website: <http://www.jobs.uconn.edu>**. The University of Connecticut is an Equal Employment Opportunity/Affirmative Action Employer.

ASSISTANT PROFESSOR POSITION in Epigenetics at The University of Georgia

The Department of Genetics and the Department of Biochemistry and Molecular Biology at the University of Georgia (UGA) invite applications at the Assistant Professor level for a tenure-track faculty position in epigenetics research. Candidates may work in any area of animal epigenetics research. Candidates who are interested in applying their work to aspects of obesity in connections with UGA's Obesity Initiative are especially encouraged to apply.

The successful candidate will hold a Ph.D. or equivalent in a relevant field, have postdoctoral experience, be expected to maintain a rigorous, externally funded research program, and contribute to undergraduate and graduate teaching. For information about the departments, see **websites: <http://www.genetics.uga.edu>** and **<http://www.bmb.uga.edu/home/>**. The successful candidate may be appointed in either department, based on their training and interests.

Applications must be submitted electronically as a single PDF file that includes a cover letter, curriculum vitae, and brief statements of research and teaching interests to **e-mail: genetics@uga.edu**. Three letters of recommendation should be electronically sent separately as PDFs to **e-mail: genetics@uga.edu**, though we will also accept hard copies mailed to: **The Genetics Search Committee, Department of Genetics, Davison Life Sciences Building, University of Georgia, Athens, GA 30602-7223**. Review of applications will begin on October 15, 2012 and continue until the position is filled.

The Franklin College of Arts and Sciences, its many units, and the University of Georgia are committed to increasing the diversity of its faculty and students, and sustaining a work and learning environment that is inclusive. Women, minorities and people with disabilities are encouraged to apply. The University is an Equal Employment Opportunity/Affirmative Action Institution.

The Department of Chemistry and Biochemistry at the University of Colorado Boulder seeks to hire a tenure-track **ASSISTANT PROFESSOR** in the area of organic chemistry to begin in the fall of 2013. The position is in the area of organic chemistry with an interest in synthesis broadly defined to include fields such as chemical biology, natural products chemistry, synthesis methods including catalysis, or related disciplines. To ensure full consideration, complete applications including letters of reference should be received by October 26, 2012. Review of applications will begin October 29, 2012 and will continue until the position is filled.

Applications will only be accepted electronically at **website: <https://www.jobsatcu.com>**, posting #818570.

*The University of Colorado Boulder is an Equal Opportunity Employer committed to building a diverse workforce. We encourage applications from women, racial and ethnic minorities, individuals with disabilities and veterans. Alternative formats of this ad can be provided upon request for individuals with disabilities by contacting the ADA Coordinator at **e-mail: hr-ada@colorado.edu**. See **website: <http://www.colorado.edu/ArtsSciences/overview/jobs/index.html>** for full job description.*

POSITIONS OPEN



FACULTY POSITION – EUKARYOTIC GENETICS AND GENOMICS University of Connecticut – Storrs

The Department of Molecular and Cell Biology at the University of Connecticut seeks to fill two nine-month faculty positions: one at the tenure-track **ASSISTANT PROFESSOR** level, and one at open rank, in Eukaryotic Genetics and Genomics, starting August 23, 2013 (search #2013194). We are interested in candidates who merge computational and functional genomics to study aspects of eukaryotic genetics, genomics, and/or genome biology. For details on the position, qualifications, and application instructions please visit **website: <http://www.jobs.uconn.edu>**. The University of Connecticut is an Equal Employment Opportunity/Affirmative Action Employer.

PHYSICIAN-SCIENTIST INVESTIGATOR in Gastroenterology and Hepatology Weill Cornell Medical College

The Division of Gastroenterology and Hepatology of the Department of Medicine, Weill Cornell Medical College, in conjunction with the Center for Advanced Digestive Care, New York-Presbyterian Hospital/Weill Cornell Medical Center, have two tenure-track faculty positions available for investigators. One position is for an individual engaged in fundamental laboratory research in the area of inflammatory bowel disease, and the other position is for an investigator working in the general area of hepatocyte development and biology. Candidates are expected to qualify for faculty positions at the Assistant Professor level at Weill Cornell Medical College, and to have or will have NIH funding for their research. Physician-scientists with M.D. or combined M.D.-Ph.D. degrees are preferred. Competitive startup packages and laboratory resources will be provided. Applications will be considered until November 15, 2012.

Interested candidates should send their curriculum vitae to: **Andrew I. Schafer, M.D., Chair of Gastroenterology-Hepatology Research Search Committee, c/o Beverly Borg, 525 East 68th Street, Box 130, M-522, New York, NY 10065** or to **e-mail: bborg@med.cornell.edu**.

Equal Opportunity Employer/Minorities/Females/Persons with Disabilities/Veterans.

HARVARD UNIVERSITY

The Department of Psychology anticipates hiring a **SENIOR LECTURER** to begin July 1, 2013.

The appointee will be expected to teach four courses per academic year including undergraduate and graduate courses in statistics, research design, and data analysis. In addition to expertise in standard multivariate techniques of data analysis, working knowledge of simulation techniques and potentially techniques including graph theory and Bayesian analysis would be desirable. The appointee may also be asked to serve on undergraduate senior thesis committees, dissertation committees and other departmental committees.

This appointment requires a Ph.D. and candidates will ordinarily have held a faculty position at a research university or a selective undergraduate institution. Candidates can submit application materials online at **website: <https://academicpositions.harvard.edu/postings/4305>**. Questions regarding this position at Harvard may be sent to **e-mail: jenniferwalker@fas.harvard.edu**. The closing date for applying is December 1, 2012.

This appointment is renewable every five years based on performance and curricular needs. Harvard is an Affirmative Action/Equal Opportunity Employer and welcomes applications from women and members of minority groups.

POSITIONS OPEN



CHAIR of The Department of Biology Colorado State University

The Department of Biology in the College of Natural Sciences at Colorado State University has an open search for the Chair of the Department. Candidates who hold a Ph.D. in Biology or an allied field, with (1) a proven record of excellence in research and teaching, (2) evidence of effective leadership, and (3) eligibility to hold the rank of **FULL PROFESSOR** at a Carnegie RU/VH institution are encouraged to apply. This is a 12-month, full-time position with salary and other forms of support commensurate with qualifications. For full consideration, applications (which includes four documents: a letter of intent, statement of leadership experience and skills pertaining to research, teaching, and outreach, a current curriculum vitae, statement of research, and list of references) should be combined into one PDF document and submitted electronically via **website: <http://cns.natsci.colostate.edu/employment/BiologyChair>** by November 15, 2012. Application materials, including letters of recommendation of semifinalist candidates will be made available for review by the Biology Faculty. The full job description can also be found at **website: <http://cns.biology.colostate.edu/employment>**.

Inquiries should be addressed to: **P. Shing Ho, Chair, Biology Chair Search Committee, College of Natural Sciences, Colorado State University, Fort Collins, CO 80523-1801, e-mail: shing.ho@colostate.edu**.

CSU is an Equal Opportunity/Equal Access/Affirmative Action Employer. Colorado State University conducts background checks on all final candidates.

ECOLOGIST/EVOLUTIONARY BIOLOGIST

The Biology Department of Franklin & Marshall College invites applications for three one-year **VISITING ASSISTANT PROFESSOR** positions in ecology and evolutionary biology, beginning July 2013 (pending administrative approval). Candidates should have the Ph.D., demonstrated strengths in teaching and research in field and/or laboratory settings, and broad interests in ecology and/or evolutionary biology. Teaching responsibilities will include lectures and laboratories in an evolution-centered introductory course that includes basic Mendelian genetics and ecology, and an upper-level lecture/laboratory course in the candidate's area of specialization. Applicants with the ability to teach introductory biostatistics will be preferred. The successful candidates will have the opportunity to engage undergraduates in research, and participate in our interdisciplinary major programs, including Environmental Studies/Science and Biological Foundations of Behavior (neuroscience and animal behavior). Franklin & Marshall is a small (enrollment 2400), highly selective coeducational liberal arts college with a tradition of excellence in science and student research.

Applicants should arrange to have letters sent from three referees, and should submit curriculum vitae, cover letter, plans for actively engaging undergraduates through teaching, teaching evaluations (if available), and undergraduate and graduate transcripts. Electronic applications will not be accepted. Priority will be given to completed applications received by November 9, 2012. Send applications to: **Dr. Daniel Ardia, Department of Biology, Franklin & Marshall College, P.O. Box 3003, Lancaster, PA, 17604. Telephone: 717-291-4118; fax: 717-358-4548; e-mail: janice.kaufman@fandm.edu; website: <http://www.fandm.edu/biology>**. Franklin & Marshall College is committed to having an inclusive campus community, and as an Equal Opportunity Employer, does not discriminate in its hiring or employment practices on the basis of gender, race or ethnicity, color, national origin, religion, age, disability, family or marital status, or sexual orientation.



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POSITIONS OPEN

MOLECULAR MICROBIAL ECOLOGIST UNC Charlotte

The Department of Biology at the University of North Carolina at Charlotte is seeking applicants for a tenure-track **ASSISTANT PROFESSOR** position. Applicants should possess a Ph.D. in the biological sciences with expertise in the area of Molecular Microbial Ecology. Candidates are expected to document expertise in their specialty by a record of postdoctoral training, peer-reviewed publications, and plans for supporting an extramurally funded research program. While being open to work with any suitable microbial system, higher consideration will be given to applicants that complement existing expertise in aquatic microbial ecology, including environmental health. Previous teaching and mentoring experience is highly desirable.

The successful candidate is expected to contribute to teaching and mentoring in our undergraduate and graduate curricula with a focus on Molecular Microbiology in an ecological context. The Department of Biology supports B.S., B.A., M.S. (thesis and non-thesis) and Ph.D. programs with a diverse body of faculty and students and prides itself with hands-on training of its students. The Department and College strongly support and value diversity among their students and faculty.

Candidates must apply online at **website: <http://jobs.uncc.edu>**, position number: 6898. Please provide a complete curriculum vita, philosophy statements for research and teaching in an ethnically diverse environment, contact information for three references, and three representative publications. Screening of applications will begin November 12, 2012, and continue until the position is filled. The expected start date is August 15, 2013.

For more information, please contact **Dr. Matt Parrow** (e-mail: mwparrow@uncc.edu), Search Committee Chair, and/or the Biology Department **website: <http://biology.uncc.edu>**.

The University of North Carolina at Charlotte is an Equal Opportunity Employer/Affirmative Action Employer and an ADVANCE Institution that strives to create an academic climate in which the dignity of all individuals is respected and maintained. Therefore, we celebrate diversity that includes, but is not limited to ability/disability, age, culture, ethnicity, gender, language, race, religion, sexual orientation, and socio-economic status.

WORKSHOP: ENVIRONMENTAL PROTEOMICS

December 10-14, 2012

Environmental Proteomics Laboratory,
California Polytechnic State University,
San Luis Obispo, California

The five-day workshop will provide a hands-on introduction to proteomics methodologies, specifically 2-D gel-electrophoresis and mass spectrometry for the identification of proteins. The workshop will focus on optimizing the analysis of the proteome of non-model and model organisms that are of interest to ecophysicologists. We will provide a detailed introduction into sample preparation techniques, 2-D gel-electrophoresis, 2-D gel image analysis, mass spectrometry, bioinformatics to process and analyze spectra, and tools to interpret proteomic results. Participants are encouraged to analyze their own samples during the workshop. Travel costs, accommodations and per diem up to \$1,575 per person will be covered through National Science Foundation grant 1253059 (**website: <http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=1253059>**). The course is open to advanced undergraduate and graduate students, postdoctoral fellows, and faculty. There are no prerequisites other than an interest in applying proteomics to an ecological or evolutionary question. Please send curriculum vitae with a research statement included and an essay explaining the reasons for wanting to participate in the workshop to **Dr. Lars Tomanek** (e-mail: ltomanek@calpoly.edu) by October 31, 2012. For more details about the laboratory, see **website: <http://www.calpoly.edu/~bio/EPL/index.html>**.

POSITIONS OPEN

CELL/MOLECULAR BIOLOGIST

The Department of Biological Sciences, University of Denver (DU), invites applications for a Cell or Molecular Biologist in a tenure-track position at the **ASSISTANT PROFESSOR** level to begin September 1, 2013. Candidates with research interests in the broad fields of development, cellular biophysics, cell signaling, and neuroscience are particularly encouraged to apply. The successful candidate will have a Ph.D. and postdoctoral experience in appropriate fields, will develop a strong extramurally funded research program, will supervise graduate and undergraduate research projects, and teach introductory courses in one of the following areas: cell biology, genetics, or physiology, as well as upper level courses in specific areas of expertise. All candidates must submit their application through **website: <https://www.dujobs.org>**. The online application should include: curriculum vitae, statements of research interests and teaching philosophy, and copies of two recent publications. Under a separate cover send three letters of recommendation to: **Dr. Scott Barbee, Chair, Cell/Molecular Biologist Search Committee, Department of Biological Sciences, University of Denver, Denver, CO 80208**. Review of applications will begin November 1, 2012. *The University of Denver is committed to enhancing the diversity of its faculty and staff and encourages applications from women, minorities, people with disabilities and veterans. DU is an Equal Employment Opportunity/Affirmative Action Employer. Please see our extensive benefit package at [website: <http://www.du.edu/hr/benefits>](http://www.du.edu/hr/benefits).*

FACULTY POSITIONS in Marine Chemistry/Geochemistry

The Skidaway Institute of Oceanography invites applications for two faculty positions at the **ASSISTANT PROFESSOR** level in Trace Element Geochemistry and Marine Environmental Chemistry. Applications from more senior candidates will also be considered. The successful candidate must have a Ph.D. and is expected to develop an active, extramurally funded research program. We are particularly interested in a collaborative colleague who can demonstrate experience in conducting field-based, interdisciplinary research in estuarine, coastal, and/or marine environments. Pertinent areas of research include, but are not limited to:

Marine Environmental Chemistry: aquatic toxicology; and fate, reactivity, transport and impact of organic compounds in coastal systems.

Trace Element Geochemistry: estuarine processes (including those associated with rivers and/or groundwater), sediment-water interactions, use of trace elements to examine coastal and continental shelf processes (chemical, geological, and/or biological), and trace element contaminants.

The successful applicant will also teach at the undergraduate and graduate level in joint educational programs with other University System of Georgia institutions. We seek faculty whose interests complement existing strengths in marine chemistry and biogeochemistry, microbial ecology, marine geology and physical observations and modeling. Located near Savannah, Georgia, the Skidaway Institute of Oceanography provides direct access to and infrastructure for research in extensive salt marsh, estuarine, and continental shelf environments. Send curriculum vitae, statement of research interests and teaching experience and contact information for at least three references, or direct inquiries to either **e-mail: trace_element_geochemist@skio.usg.edu** or **environmental_chemist@skio.usg.edu**. Please attach required documents in PDF format. Electronic applications are strongly encouraged, but paper applications may be sent to: **Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, GA, 31411**. Review of applications will begin on November 12, 2012. *Equal Opportunity Employer/Affirmative Action Employer.*

POSITIONS OPEN

ASSISTANT PROFESSOR - CLIMATE Massachusetts Institute of Technology

The MIT Department of Earth, Atmospheric and Planetary Sciences has been undergoing a major expansion of its activities in climate science. Positions have recently been filled in the areas of Atmospheric Chemistry and Paleo Climate. We seek applicants for further appointments in Climate-related fields as they pertain to: observations, models and theory of the ocean and cryosphere, and related biogeochemical cycles and ecology. Preference will be given to junior appointments at the **ASSISTANT PROFESSOR** level, but a senior appointment can be considered for an individual with exceptional qualifications.

The successful candidates will have a strong record of accomplishment in their discipline, a strong commitment to teaching and student advising, and a keen interest in relating their work to complementary research in the Department and in the MIT/Woods Hole Joint Program in Oceanography. Joint appointments with other MIT departments are also potentially negotiable where appropriate.

Applicants should submit curriculum vitae; a one-page description of research and teaching plans; and the names, e-mail addresses, and telephone numbers of three professional referees. Please do not ask your referees to upload letters at the time of application; letters will be requested directly by MIT. Questions may be addressed to **Professor John Marshall**, Search Committee Chair, at **e-mail: jmarsh@mit.edu**. Applications are being accepted at Academic Jobs Online, **website: <https://academicjobsonline.org/ajo>**. To receive consideration, a complete application must be received.

Search Contact: **Mr. Michael Richard, Human Resources Administrator, EAPS, 54-912 Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139; e-mail: mjr@mit.edu; telephone: 617-253-5184; fax: 617-253-8298**.

MIT is an Equal Opportunity/Affirmative Action Employer; applications from women and underrepresented minority candidates are encouraged. MIT is a nonsmoking environment.

Bowdoin College invites inquiries, nominations, and applications for the newly endowed position of **DIRECTOR** of the Bowdoin College Marine Laboratory and **PROFESSOR** of Biology. We seek an established scholar with a record of excellence in teaching and research, with a demonstrated track record of extramural funding and mentoring of undergraduate research. All fields of marine biology. Successful candidate will establish a research program at Bowdoin's flowing seawater marine lab.

Bowdoin's Marine Lab is located at the Coastal Studies Center on Orr's Island, approximately 8 miles from the Bowdoin campus (**website: <http://www.bowdoin.edu/coastal-studies-center>**). The College offers competitive salaries and startup packages, a 2-1 teaching load, small classes, a culture of student research, and excellent research and grant support infrastructure.

Nominations and expressions of interest may be directed to **Cristle Collins Judd**, Dean for Academic Affairs at **e-mail: cjudd@bowdoin.edu**.

Visit **website: <http://www.bowdoin.edu/about/employment/index.shtml>** to apply.

Review of applications will begin will begin October 19, 2012.

Bowdoin College is committed to equality through Affirmative Action, and is an Equal Opportunity Employer.

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Nominations are open **now through October 15** for the **AAAS Early Career Award for Public Engagement with Science**.

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FOCUS ON CAREERS

Focus on China: A Tale of Two Cities

In This Issue

As the Chinese government invests increasing amounts of money into scientific research, and biotech multinationals rush to open offices in China, opportunities for scientists are exploding. Two cities stand at the forefront of Chinese science. Beijing, close to the center of power, offers more in terms of academia, government jobs, and research. By contrast Shanghai, China's commercial hub, is home to the lion's share of biotech. China is a land of diverse opportunities and myriad challenges; from those already there, objectives, ideas, and advice vary. One thing is certain: The world's most populous nation and second-largest economy is fast becoming a global hub for scientists of the future.

See full story on page 1692

Upcoming Features

Neuroscience Careers—October 5

Top Employers Survey (print edition)—October 19

European Regional Focus—November 9

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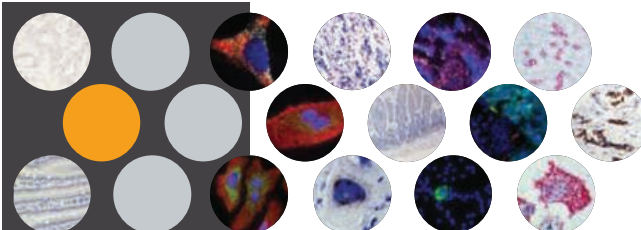
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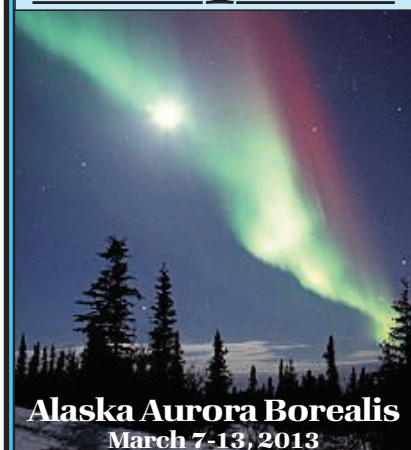
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New Products

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The Spectroline CL-150 Series UV workstations enable lab technicians to view, analyze, and photograph fluorescent samples with both epi-illumination and transillumination light sources. A removable bottom panel allows either unit to be placed directly over the transilluminator. The 25 W white light bulb in the cabinet provides visible illumination for easy sample preparation. Researchers can either utilize the safety viewing eyepiece or substitute a “snap-on” camera adapter for photography. Simply place the adapter onto the cabinet in place of the eyepiece to produce superior quality digital images. The spacious CL-150 cabinet is able to accommodate large electrophoresis gels and up to four 8 in × 8 in (20 cm × 20 cm) TLC plates. It can be used with any one or two of 11 different models of Spectroline X- and XX-series 15 W UV lamps (separately available).

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ICE BUCKETS AND ICE PANS

In addition to their superior insulation properties and chemical resistance, Magic Touch 2 ice buckets and ice pans include a convenient pour spout to easily drain unwanted ice melt, drip-proof rims that keep the bench dry, and snug-fitting, keyed lids and bases for easy stacking. The new Magic Touch 2 Icewares are compatible not only with wet ice, but also with ultralow temperature materials including dry ice, salt slurries, dry ice solvent slurries, or liquid nitrogen. Able to withstand a wide temperature range of -196°C (-320°F) to 100°C (212°F), these high quality, expanded urethane icewares are lightweight, durable, nonsweating, and impervious to moisture and odors. Scienceware Magic Touch 2 ice buckets are available in 2.5 L and 4.0 L sizes with lids. Ice pans are available in 1.0 L, 4.0 L, and 9.0 L sizes with lids, and in the 9.0 L size without lid. All are available in four vibrant new colors: ebony, true blue, cherry red, and emerald green.

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A broad lineup of open tank circulating bath systems for laboratory use are available with either stainless-steel or polycarbonate tanks. These economical baths are offered in reservoir sizes ranging from 6 to 28 L and with three different temperature controllers, including a programmable model with a highly intuitive operator interface. All controllers display both actual and setpoint temperature on a read-out that is easily read from across the lab. These stainless-steel open tank systems are available with 6, 10, 20, or 28 L reservoirs and either the Advanced Programmable, Advanced Digital, or MX controller. Polycarbonate models feature 8, 11, 14, 17, 23, or 28 L reservoirs and come with either the Advanced Programmable or MX controller. All models feature an insulating, chemical resistant top deck that remains cooler at high temperatures and is easily cleaned and disinfected.

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WEBINAR

Advancing Epigenetics: Novel Drug Discovery Strategies for Epigenetic Targets

Wednesday, October 17, 2012

12 noon ET, 9 a.m. PT, 4 p.m. GMT, 5 p.m. UK

Research in the field of epigenetics has exploded in the last several years, as evidenced by the rapidly expanding volume of published literature. Significant advances have been made in the illumination of basic epigenetic mechanisms such as chromatin remodeling, DNA methylation, and RNA signaling. With these advances has also come an understanding of the critical roles that epigenetic modifications play in the development and progression of disease, and an increased focus on the identification of clinical treatments for aberrant epigenetic states. Given the increasing interest and investment in drug discovery for epigenetic targets, there is a clear need for robust methodologies to identify candidate therapeutic compounds. This webinar will focus on novel techniques for drug discovery against histone-modifying enzymes.

During this webinar, our panelists will:

- Briefly cover the basics of epigenetic modifications and how they impact gene expression and disease states
- Discuss current techniques for identifying and validating potential targets for clinical intervention
- Share their personal experience working with histone-modifying enzymes as therapeutic targets.
- Answer your questions live during the broadcast!

Participants:

Manfred Jung, Ph.D.
University of Freiburg
Freiburg, Germany

Margaret Porter Scott, Ph.D.
Epizyme
Cambridge, MA

Ji-Hu Zhang, Ph.D.
Novartis Institutes for
Biomedical Research
Cambridge, MA

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